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VOL. XVIII, PART I

(March 1948)

The Publication Committee of the Indian Council of Agricultural Research ndia, takes no responsibility for the opinions expressed in this Journal

Vol. XVIII, Part 1 March, 1948

The Indian Journal of Veterinary Science and Animal Husbandry

Issued under the authority
of
The Indian Council of Agricultural Research

Annual subscription
Rs. 6 or 9s. 6d.

Price per part
Rs. 2 or 3s.

PUBLISHED BY THE MANAGER OF PUBLICATIONS, DELHI
PRINTED BY THE GOVERNMENT OF INDIA PRESS, CALCUTTA, INDIA,
1948

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VOL. XVIII, PART II

(June, 1948)

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Vol. XVIII, Part III

(September, 1948)

Vol. XVIII, Part III September 1948

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Published by the Manager of Publications, Delhi
Printed by the Government of India Press, Calcutta, India,
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VOL. XVIII, PART IV

(December 1948)

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Original articles

affected and the heaviest individual district mortality figures were 1,000 in 24-Parganas, 414 in Jalpaiguri, 100 in Khulna and 97 in Chittagong. In 1918-19, only five districts were affected and the bulk of the mortality recorded was in 24-Parganas which returned a figure of 2,503 deaths. During these two years, this district lost over 3,500 cattle from anthrax—the heaviest mortality ever experienced in any district of Bengal. Moreover, in 1918-19 mortality due to anthrax constituted as much as 16·8 per cent of the total mortality in bovines due to contagious disease. For 12 years after this, the mortality figures of the Province varied between 63 and 262. In 1931-32, 517 deaths were recorded from 14 districts, the heaviest affected being Burdwan (111) and Chittagong (99). In 1932-33, there were 309 deaths in eight districts (Dacca, 114 ; Bakarganj, 92) and in 1933-34, 329 deaths were recorded from 11 districts (Mymensingh, 86 ; Rangpur, 60). Then followed a period of comparatively heavy mortality extending over eight years from 1934 to 1942. The first year of this period, viz., 1934-35, recorded 1,437 deaths from 14 districts (Khulna, 1,167). In 1935-36, there were 677 deaths in 15 districts (Dacca, 297 ; Pabna, 104 ; Noakhali, 76). From 1936-37 to 1941-42, the figures varied between 1,027 (1940-41) and 1,873 (1937-38), the heaviest losses occurring in Dacca (2,956 during 1935-1940), Mymensingh (606 during 1936-1940) and Tippera (497 during 1937-1940). In 1939-40, as many as 22 districts were affected. Afterwards, the mortality figures gradually came down from 478 in 1942-43 to 320 in 1944 45.

Percentage of mortality

In individual outbreaks in cattle, the percentage of deaths reported, has occasionally been as low as 40 to 50 seldom lower, and it has often been as high as 100 per cent in outbreaks of different sizes. During 1898-1904, there was a total of 1,532 deaths out of 2,044 seizures, the percentage mortality working to 75 and, in individual years, varying between 67 and 78. Balwant Singh [1937] observed a mortality of 70 per cent during the East Bengal epizootic of 1936. During recent years (1937-1945), about 85 per cent deaths have been recorded, with a variation in the annual figures of 75 to 90 per cent.

Size of an outbreak

It is not always easy to decide what constitutes an outbreak. This is especially so in the case of anthrax, because sometimes each and every case may be a separate outbreak. For purposes of reporting, it is customary with the district staff, to consider each affected village as a separate outbreak, but when any contagious disease is reported to them as having broken out in more than one village on the same date, they regard all these affected villages as falling under one outbreak. Wherever possible in this paper, each affected village has been regarded as a separate outbreak. Very often, outbreaks have been limited to one, two, or very few animals. Sometimes, up to fifty animals, or even more, have been reported to be affected in one outbreak. Analysis of nearly one thousand outbreaks which occurred during 1937-1946 gives an average of roughly eight deaths per outbreak. Considering the system of reporting in vogue and also the probability of many single-death-outbreaks going unrecorded, this figure would seem rather high. This, of course, does not cover the possibility of clinically inapparent infections occurring to any extent.

Species affected

In Bengal, as in most other places, the bulk of the mortality due to anthrax occurs in cattle which also constitute the bulk (over 70 per cent) of the total livestock population of the Province.

Buffaloes are not known to be affected to an extent proportionate to their number—over one million and roughly 4·5 per cent of all bovines—and have often been noticed to escape infection. During the seven years from 1936-37 to 1942-43, anthrax is reported to have killed only 186 buffaloes against 8,659 cattle (*i.e.*, a proportion of one buffalo to every 46 cattle and an average of roughly 27 buffaloes a year) and to have caused approximately 1·4 per cent of all deaths in buffaloes (13,335) during this period.

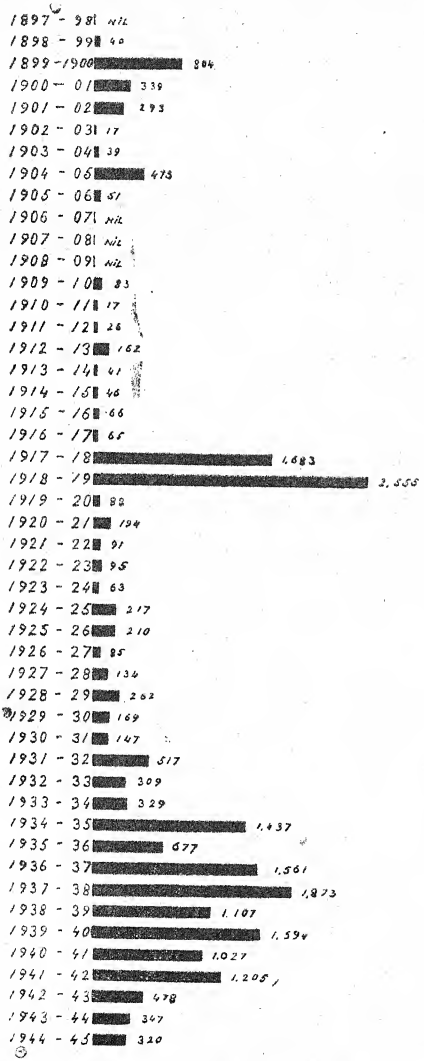

FIG. 1. Mortality due to anthrax in Bengal.

There have been deaths among the equines practically every year, but the mortality figures have always been very low. The total mortality due to anthrax among equines during 30 years from 1911-12 to 1942-43 is 87 (or 3·2 per cent) out of a total mortality in them due to contagious diseases of 2,697, and the maximum number of deaths occurring in any year was 15 in 1917-18. This is perhaps largely due to the fact that Bengal is not a horse-raising country ; the total equine census of this Province is 77,398 only.

In sheep, only four outbreaks have been recorded so far, mostly from Dacca. The mortality caused was two in 1938-39, 32 in 1936-37, 49 in 1932-33, and 20 in 1923-24.

In goats, especially from 1936-37 onwards, outbreaks have been reported from some districts, the annual mortality varying between 20 and 154. The total mortality caused by anthrax in goats from 1936-37 to 1941-42 was 377 (or 2 per cent) out of a total mortality of 17,506 from all causes in this species, and about 54 per year on an average.

In 1932-33, one death due to anthrax in an elephant was recorded at Jalpaiguri. There are no authentic reports of the disease breaking out in pigs and other susceptible species.

Anthrax in man

There have been occasions when the disease has spread to human beings. In the 1936 epizootic [Kerr and Balwant Singh, 1936] involving certain districts in East Bengal, 66 human beings (58 males and 8 females) became affected. These people were either directly or indirectly connected with slaughter of infected animals and handling of their hides and flesh. They were mostly hide-merchants, butchers and cultivators and belonged to beef-eating communities.

In one of the affected villages (Shayestapur, District Noakhali) a cultivator slaughtered a sick cow which he had purchased the previous day from a neighbouring market, sold, and distributed its meat to other villagers. Thirteen persons who handled this meat contracted the infection and two of them, both women, died. Four cattle and one goat also became affected.

The malignant pustules, developing in human beings in these outbreaks generally remained localized and the majority of the affected persons recovered completely in a few weeks to a few months. In eight, however, septicaemia developed and death resulted in three to seven days. The pustules were located on different parts of the body, those most commonly affected being the hands, especially the fingers, and the face. In order of frequency, the upper extremities were involved in 45 per cent of cases, the head and face in 27 per cent, the legs in 20 per cent and the trunk in 8 per cent. In five of the eight fatal cases, the pustules were on head and face, in two on the fingers, and in one on the leg.

Age and sex of affected animals

There are no records to show at what age the different species of animals acquire infection in largest number. It seems, however, that adult animals about the middle part of their life, say three to six years in the case of cattle, are those most susceptible. Regarding sex, there is absolutely no information. It is possible to make a general statement that so far no particular age, sex, breed and colour susceptibility to the disease has been observed.

Clinical aspects

In this paper, it is not proposed to deal at length with clinical symptoms and post-mortem features. Reference was made to the symptoms described by Raymond (*loc. cit.*) for ' Loodiana disease ' as well as those briefly noted by Kerr and Balwant Singh (*loc. cit.*). Some information was also collected from certain officers of the Department, who claimed first-hand clinical experience of the disease in Bengal. All these accounts were, in general, found to correspond fairly with the standard text book descriptions of the disease and the variety of symptoms commonly observed in other parts of the world.

It may, however, be noted that in goats, the disease has generally been seen to run a peracute course, proving fatal in almost all cases. Deaths have been noted to occur in a few hours and, in some cases, even in a few minutes after the onset of symptoms.

DIAGNOSIS

Since some years ago, it has been made the routine practice for the district staff to submit for microscopical examination blood-films from cases suspected of anthrax. Very often, however, neither any affected animals nor any carcases are available at the time the Veterinary Assistant Surgeon visits the affected village, so that, not all outbreaks reported as anthrax are microscopically confirmed. Examination of some 200 outbreaks which occurred during the latter part of the period after 1940 showed that only about 60 per cent of these were confirmed.

In most parts of Bengal, the cattle owners are not so familiar with anthrax as they have become with diseases like rinderpest, foot-and-mouth disease, black-quarter and haemorrhagic septicaemia. Most of them fail to recognize anthrax, especially at the beginning of an outbreak, when even competent technical officers, and especially those who have had no previous experience of the disease, may often find it difficult to diagnose correctly from clinical signs alone. It seems probable that anthrax is often mistaken for other diseases such as haemorrhagic septicaemia, poisoning, snake bite, etc. Added to these difficulties is the fact that the disease usually occurs sporadically in well-separated localities.

Many of these points are repeatedly stressed in the annual reports of the Department and, in view of this, a hundred per cent reliance cannot be placed on the recorded figures.

Seasonal prevalence

In the war against any disease, knowledge of its natural behaviour is always helpful. An important aspect of this is the season to season variation in its incidence. Moreover, when dealing with a vaccine, the immunity conferred by which is not expected to last for more than a few months, it becomes essential to determine at what time of the year the prophylactic inoculations should be undertaken so that revaccination may not become necessary until the following year.

For securing information about the seasonal prevalence of anthrax in Bengal, records giving figures of outbreaks in different months in different districts of the Province during the ten year period from 1937 to 1946 were examined. They were also analysed statistically.

These figures (Tables I and II) have shown that the outbreak incidence curve in Bengal is at a low point in January. It shows a tendency to rise in February and March, followed by a somewhat sudden jump to very near the peak in April. The peak is reached during May and June, but during July and August the curve declines, reaching its almost lowest point of the year in September. Again, in October and November, there is an upward tendency, before the final decline to the lowest point of the year in December. Among the different districts, there seems to be no appreciable and significant difference in the seasonal behaviour of these outbreaks, beyond some minor irregular variations.

TABLE I

Monthly incidence of anthrax outbreaks in Bengal during years 1937-1946

Year	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946	Total
Month											
January .	6	10	3	6	..	6	4	2	1	2	40
February .	5	8	5	11	20	5	1	..	..	2	57
March . .	6	9	7	8	8	9	2	5	..	3	57
April . .	35	13	31	15	36	14	2	6	4	3	159
May . .	40	27	32	10	48	12	3	1	3	7	183
June . .	29	30	43	11	32	17	3	5	5	3	178
July . .	38	7	22	17	18	11	10	8	2	..	133
August . .	12	7	8	4	18	5	2	2	1	..	59
September .	10	5	6	2	1	2	3	..	1	1	31
October .	3	9	1	6	9	8	..	..	..	..	36
November .	7	5	1	3	12	5	5	..	..	1	39
December .	3	7	6	4	2	1	1	2	..	..	26
TOTAL .	194	137	165	97	204	95	36	31	17	22	998

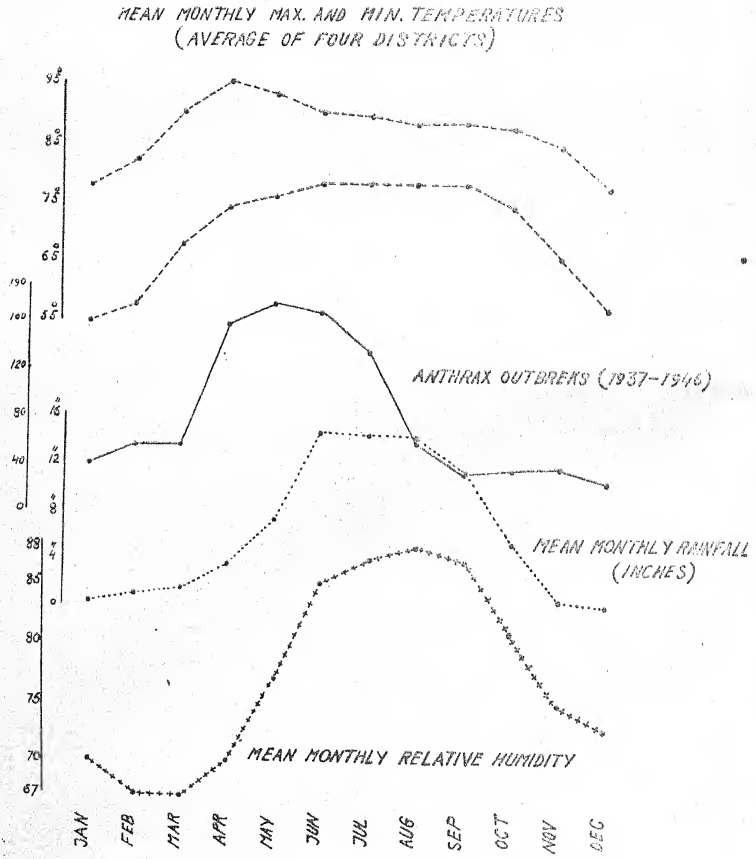

FIG. 2. Relation between incidence of anthrax and weather conditi...

TABLE II

Monthly incidence of anthrax outbreaks in the districts of Bengal during 1937-1946

Serial No.	District	Jan.	Feb.	March	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
1	24-Parganas	..	..	4	12	10	2	8	2	1	1	..	..	40
2	Howrah	..	..	..	..	..	1	..	..	..	..	..	..	1
3	Midnapore	1	11	10	19	22	22	9	7	6	5	2	..	114
4	Nadia	..	..	..	..	..	..	..	..	..	..	..	1	1
5	Murshidabad	5	4	1	1	3	7	9	1	2	2	7	2	44
6	Jessore	..	..	1	..	..	..	..	..	..	..	..	1	2
7	Khulna	1	..	..	10	7	2	1	4	..	..	..	..	25
8	Hooghly	..	..	2	1	..	..	..	1	..	..	..	..	4
9	Bardwan	2	..	1	2	1	2	4	4	3	..	1	3	23
10	Birbhum	2	..	..	..	6	6	2	1	2	..	3	1	23
11	Bankura	1	..	..	1	5	2	4	3	1	..	1	..	18
12	Darjeeling	..	..	1	1	..	1	1	1	1	..	..	..	6
13	Jalpaiguri	..	..	1	6	4	3	..	2	..	..	1	2	19
14	Rangpur	..	3	7	12	8	4	9	3	..	2	1	2	51
15	Cooch-Behar	1	..	..	5	4	2	1	..	1	..	..	1	15
16	Rajshahi	2	1	..	4	1	1	1	1	..	1	2	..	14
17	Pabna	2	..	..	5	..	9	6	2	..	1	2	..	27
18	Bogra	..	..	1	3	7	9	6	1	2	..	1	2	32
19	Dinajpore	1	1	1	1	7	5	2	3	..	5	5	1	32
20	Malda	..	1	..	1	..	..	..	3	1	..	1	..	7
21	Mymensingh	1	10	4	22	19	25	19	7	2	5	1	6	121
22	Dacca	10	15	6	20	17	49	37	11	6	9	9	2	191
23	Faridpore	..	..	1	..	1	1	5	..	..	2	..	..	10
24	Bakarganj	1	5	6	6	6	10	3	1	1	1	..	..	40
25	Chittagong	2	..	..	7	2	2	..	..	2	..	..	..	15
26	Ch. H. Tracts	..	..	1	4	6	1	1	..	..	..	..	..	13
27	Tippera	4	1	8	12	37	6	3	..	..	2	2	1	79
28	Noakhali	4	2	1	4	10	6	2	1	..	..	..	1	31
	TOTAL	40	57	57	159	183	178	133	59	31	36	39	26	998

It may be concluded from this that while anthrax may break out in any part of Bengal at any time of the year, the majority of the outbreaks occur during the summer months. Thus, of the 998 outbreaks which occurred during 1937-1946 more than 65 per cent were during the four months of April, May, June and July. Analysis of the seizure and mortality figures for this period has also revealed a very much higher incidence of the disease in these four months than in the other months.

It is generally believed that in warm countries the maximum incidence of anthrax is in the months of June, July, August and September [Topley and Wilson, 1936]. The present study, however, shows that the period of maximum incidence in Bengal slightly precedes this, it being the summer and the first half of the rainy season.

On comparing the incidence curve of anthrax outbreaks in Bengal with the prevailing weather conditions the two factors which appear to mainly coincide with this incidence are the atmospheric temperature and the rainfall, probably as they affect the soil and other environmental conditions. Thus, in December and January (Tables I and II), when the weather is cool and dry and the rainfall is at its minimum, the incidence of anthrax outbreaks is very low. In February and March, when both maximum and minimum temperatures rise, rainfall increases a little (though the humidity is still low), the incidence, though still rather low, shows a tendency to increase. In April, when the temperature rises to very near the most optimum temperature for vegetation and multiplication of *B. anthracis*, rainfall increases a little more, and the humidity, though still low, shows a tendency to rise, the incidence increases considerably. In May, when the mean temperature is about the same as in April, the rainfall increases considerably and the humidity also rises, the incidence is at its highest. In June, when the maximum temperature comes down and the minimum temperature rises, both by a couple of degrees, the rainfall is heavy and the humidity high, the incidence is almost as high as in May. In July, when the temperature is still favourable, humidity very high and the rainfall heavy, the incidence is moderately high but on the decline. In August, when the temperature is almost the same as in July, humidity a little higher still and the rainfall continues to be heavy, the incidence decreases considerably. In September, when the temperature and humidity remain about the same as in July and August, and the rainfall decreases a little, the incidence is almost at its lowest point in the year. In October and November, when the temperature, though gradually coming down, is still favourable, humidity about the same as in June, cloudiness and rainfall further decreased and the soil comparatively less soaked with water than before, there is a small increase in the incidence before the final decrease in December.

To sum up, the incidence of anthrax outbreaks in Bengal seems primarily to vary with the atmospheric temperature, being low in winter months, gradually rising with the approach of summer and reaching the peak when the mean temperature approaches the most optimum temperature for the growth and multiplication of *B. anthracis*. Moderately high humidity and moderately heavy rainfall seem to favour high incidence, very heavy rainfall and very high humidity keeping it low. The great decrease in the incidence from July to September may be due chiefly to a cumulative effect of the heavy and continued monsoon showers which can be expected to wash out most of the existing soil contamination, leach out the available food elements, dilute the remaining contamination, and, by raising the atmospheric humidity and the moisture content and acidity of the soil, interfere with aerobiosis and multiplication of the organisms.

The finding, that anthrax in Bengal shows a well-defined seasonal incidence can be profitably utilized in case it is decided to adopt prophylactic vaccination in any locality. Such operations can be so timed that the most active period of the immunity conferred covers the major anthrax season. Thus, the best time to undertake prophylactic vaccination against the disease in Bengal, if done only once a year, would be the period soon after the beginning of February. As it is advised to use the vaccine as fresh as possible, its manufacture and stocking in bulk may also be adjusted according to the seasonal prevalence of the disease.

YEAR TO YEAR VARIATION IN MORTALITY

The extent of annual mortality due to anthrax as recorded in the reports of the Department from 1897-98 to 1944-45 has been briefly summarized elsewhere. It is seen that the annual figures of these 48 years show a very great variation, viz., from nil to a maximum of 2,555, working to an average of under 450 deaths per year. This variation remains almost as patent when it is considered in relation to total mortality in livestock and even after allowing for fluctuations due to seasons and random causes. The extent of variation seems too great and irregular to be satisfactorily explained as due to direct influence of any climatic or other environmental factors. The three periods which stand out more or less prominently associated with heavy mortality are considered below.

(i) The year 1899-1900 recorded 804 deaths due to anthrax against 40 in the preceding and 339 in the succeeding years, and the total mortality in the three-year period from 1899-1900 to 1901-02 was 1,436.

(ii) The heaviest annual mortality ever experienced was recorded during 1917-1919 (4,238 deaths in two years).

(iii) An extended period of eight years from 1934-35 to 1941-42 recorded an average annual mortality of 1,310 in bovines and about 50 in other animals.

The periods noted above do not bear any relationship with each other and it is difficult to imagine that the heavy mortality experienced in them was primarily related to any natural factors. It seems likely that the occurrence of large epizootics and consequent heavy mortality are chiefly due to spread of infection by means which are well appreciated by animal epizootiologists. The fact that these periods have occurred at intervals of about 18 years may not be of any significance.

Regional distribution

Some idea of the regional distribution of anthrax in Bengal can be gained by considering (i) the number of years during which anthrax outbreaks have occurred in the different districts, (ii) the mortality figures of different districts, and (iii) the number of outbreaks occurring in different districts during 1937-1946.

The past years have shown wide difference in these respects. Generally speaking, the extent to which a particular district has been affected with anthrax seems to have been largely governed by its area and livestock population. Nevertheless, even after allowing for such factors, certain districts do seem to have been affected somewhat more than the others. Dacca stands out as the most heavily affected district. Moreover, it is in this district that the disease has often spread to goats and also to human beings. The other districts in which the incidence has been comparatively high in the past few years are Midnapore and Murshidabad in West Bengal, and Tippera, Noakhali and Mymensingh, flanking the banks of the Brahmaputra in East Bengal. In the Gangetic delta in south Bengal, the districts of Khulna and Bakarganj have occasionally experienced heavy mortality—that occurring in 24-Parganas in 1917-1919 has already been noted. In the remaining districts, the incidence has been low and very irregular. The central Bengal districts have been affected the least.

USE OF SERUM AND VACCINE

Both serum and vaccine have been used in the past, the former in affected villages and the latter generally in unaffected villages in areas affected with larger epizootics of the disease. In 1936, anthrax spore vaccine was obtained from Burma and was used extensively in the field after a preliminary test for its safety. In 1937, anthrax spore vaccine manufactured at the Indian Veterinary Research Institute became available. In preliminary tests, this vaccine was found to produce in a proportion of animals inflammatory swellings at the seat of inoculation and some general systemic disturbance. Such reaction, however, was seldom alarming and the vaccine was pronounced safe for general use. From 1936-37 to 1939-40, a total of 22,466 bovines, one equine and 421 other animals (sheep and goats) were vaccinated. Anti-anthrax serum has been used every year to protect thousands of animals during outbreaks. During 1945-46, when a new brew of the spore vaccine became available from the Indian Veterinary Research Institute, in addition to routine use of serum and vaccine by the district staff, these products were specially used in two selected herds. These two tests are described below.

It may be concluded from this that while anthrax may break out in any part of Bengal at any time of the year, the majority of the outbreaks occur during the summer months. Thus, of the 998 outbreaks which occurred during 1937-1946 more than 65 per cent were during the four months of April, May, June and July. Analysis of the seizure and mortality figures for this period has also revealed a very much higher incidence of the disease in these four months than in the other months.

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The past years have shown wide difference in these respects. Generally speaking, the extent to which a particular district has been affected with anthrax seems to have been largely governed by its area and livestock population. Nevertheless, even after allowing for such factors, certain districts do seem to have been affected somewhat more than the others. Dacca stands out as the most heavily affected district. Moreover, it is in this district that the disease has often spread to goats and also to human beings. The other districts in which the incidence has been comparatively high in the past few years are Midnapore and Murshidabad in West Bengal, and Tippera, Noakhali and Mymensingh, flanking the banks of the Brahmaputra in East Bengal. In the Gangetic delta in south Bengal, the districts of Khulna and Bakarganj have occasionally experienced heavy mortality—that occurring in 24-Parganas in 1917-1919 has already been noted. In the remaining districts, the incidence has been low and very irregular. The central Bengal districts have been affected the least.

USE OF SERUM AND VACCINE

Both serum and vaccine have been used in the past, the former in affected villages and the latter generally in unaffected villages in areas affected with larger epizootics of the disease. In 1936, anthrax spore vaccine was obtained from Burma and was used extensively in the field after a preliminary test for its safety. In 1937, anthrax spore vaccine manufactured at the Indian Veterinary Research Institute became available. In preliminary tests, this vaccine was found to produce in a proportion of animals inflammatory swellings at the seat of inoculation and some general systemic disturbance. Such reaction, however, was seldom alarming and the vaccine was pronounced safe for general use. From 1936-37 to 1939-40, a total of 22,466 bovines, one equine and 421 other animals (sheep and goats) were vaccinated. Anti-anthrax serum has been used every year to protect thousands of animals during outbreaks. During 1945-46, when a new brew of the spore vaccine became available from the Indian Veterinary Research Institute, in addition to routine use of serum and vaccine by the district staff, these products were specially used in two selected herds. These two tests are described below.

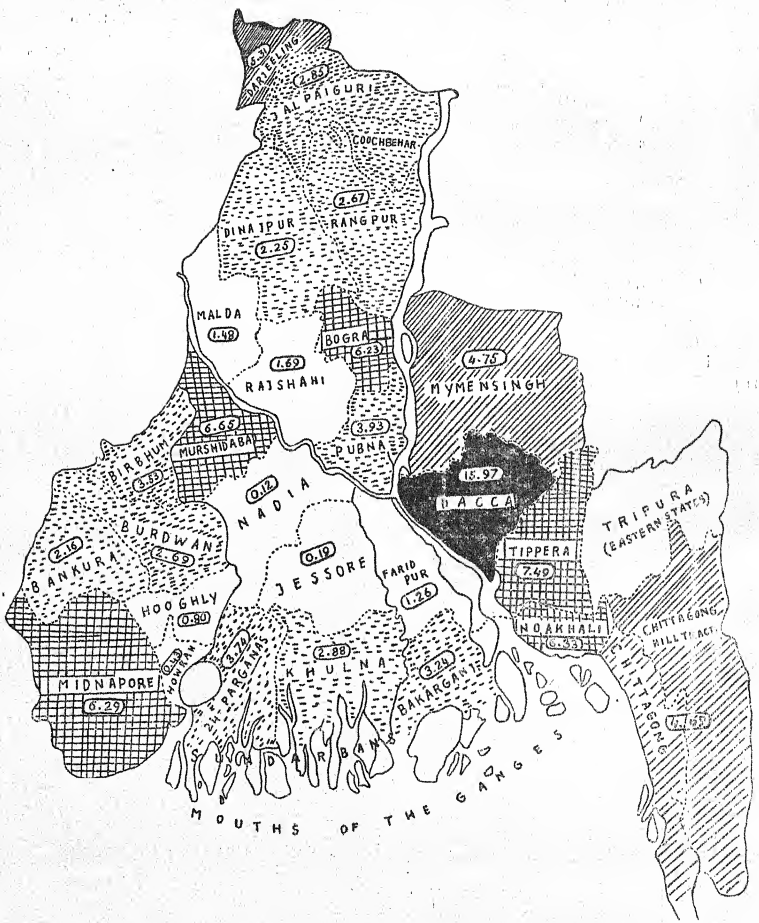

FIG. 3. Regional distribution of anthrax in Bengal—outbreaks per 100,000 bovines during 1937—1947.

TABLE III

Incidence of anthrax outbreaks in the districts of Bengal during years 1937 to 1946

Serial No.	District	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946	Total
1	24-Parganas	5	1	17	1	..	2	7	2	..	5	40
2	Howrah	..	..	..	1	..	..	..	..	..	..	1
3	Midnapore	8	7	18	14	37	10	2	9	5	4	114
4	Nadia	1	..	..	..	..	..	..	..	..	..	1
5	Murshidabad	5	6	1	5	12	10	2	1	1	1	44
6	Jessore	..	1	..	..	1	..	..	..	..	..	2
7	Khulna	11	..	..	..	4	6	..	..	2	2	25
8	Hooghly	..	2	..	..	..	..	1	..	1	..	4
9	Burdwan	3	7	4	3	3	1	1	1	..	..	23
10	Birbhum	1	1	2	2	12	..	2	1	1	1	23
11	Bankura	9	2	1	2	..	2	1	..	1	..	18
12	Darjeeling	1	1	2	..	1	1	..	..	..	..	6
13	Jalpaiguri	..	2	13	..	3	1	..	..	..	..	19
14	Rangpur	6	2	4	10	14	12	1	..	1	1	51
15	Cooch-Behar	..	1	10	1	1	1	1	..	..	..	15
16	Rajshahi	1	1	2	1	4	..	3	1	1	..	14
17	Pabna	3	2	2	2	3	3	2	8	..	2	27
18	Bogra	..	..	5	1	23	..	3	..	..	..	32
19	Dinajpur	4	..	6	..	16	6	..	..	..	..	32
20	Malda	..	..	..	1	3	1	1	1	..	..	7
21	Mymensingh	8	18	26	9	49	9	..	1	1	..	121
22	Dacca	65	41	39	18	12	8	2	2	3	1	191
23	Faridpore	2	..	..	5	1	1	..	..	..	1	10
24	Bakarganj	8	14	3	4	..	9	1	1	..	..	40
25	Chittagong	3	3	4	..	..	4	..	..	..	1	15
26	Ch. H. Tracts	..	..	..	12	..	..	..	..	..	1	13
27	Tippera	37	16	5	5	4	3	4	3	..	2	79
28	Noakhali	13	9	1	..	1	5	2	..	..	..	31
	TOTAL	194	137	165	97	204	95	36	31	17	22	998

TABLE IV

Incidence of anthrax outbreaks during 1937-1946 in the districts of Bengal compared with area, bovine population, etc.

Serial No.	District	Annual rainfall (ins.)	Area (acres)	Bovine population (1940 census)	Anthrax outbreaks 1937—1946	Outbreaks per 100,000 acres	Outbreaks per 100,000 bovines
1	24-Parganas	62·93	3,192,300	1,069,209	40	1·26	3·74
2	Howrah	59·58	347,556	234,030	1	0·29	0·43
3	Midnapore	58·71	3,336,983	1,813,090	114	3·42	6·29
4	Nadia	57·36	1,847,552	847,822	1	0·05	0·12
5	Murshidabad	54·60	1,308,137	661,933	44	3·36	6·65
6	Jessore	64·93	1,865,002	1,013,245	2	0·12	0·19
7	Khulna	67·85	3,077,589	866,536	25	0·81	2·88
8	Hooghly	57·00	737,588	499,671	4	0·52	0·80
9	Burdwan	59·62	1,728,676	853,226	23	1·33	2·69
10	Birbhum	56·17	1,115,498	651,265	23	2·06	3·53
11	Bankura	55·52	1,694,288	831,526	18	1·06	2·16
12	Darjeeling	119·43	773,261	113,377	6	0·77	5·31
13	Jalpaiguri	128·63	1,957,155	688,449	19	0·97	2·85
14	Rangpore	83·89	2,308,673	1,717,223	51	2·21	2·97
15	Cooch-Behar	..	..	..	15	..	..
16	Rajshahi	56·30	1,590,764	826,027	14	0·88	1·69
17	Pabna	59·37	1,174,971	686,649	27	2·29	3·93
18	Bogra	69·15	944,325	513,958	32	3·39	6·23
19	Dinajpore	72·26	2,537,768	1,424,715	32	1·22	2·25
20	Malda	56·63	1,275,039	473,270	7	0·55	1·48
21	Mymensingh	66·98	4,033,920	2,550,488	121	3·00	4·75
22	Dacca	73·36	1,751,609	1,195,959	191	10·90	15·97
23	Faridpore	71·03	1,561,655	793,736	10	0·64	1·26
24	Bakarganj	81·21	2,302,657	1,232,815	40	1·74	3·24
25	Chittagong	107·63	1,738,569	527,007	15	0·56	4·69
26	Ch. H. Tracts	96·18	3,204,570	69,725	13		
27	Tippera	94·33	1,619,590	1,054,704	79	4·88	7·49
28	Noakhali	115·00	1,022,910	489,978	31	3·03	6·33

Provincial average—1·96 outbreaks per 100,000 acres (excluding Cooch-Behar) or 4·15 outbreaks per 100,000 bovines.

The first herd, Midnapore Central Jail dairy herd, was maintained in two separate groups, one inside the jail compound (Group A) and the other outside (Group B). All the 29 cattle of Group B (seven bullocks, two cows and 20 calves) were inoculated with serum within a week of the sudden death of a calf in this herd (heart blood-films examined in Calcutta and found positive for *B. anthracis*) and, on 20 August, the 42 cattle of Group A (one bull, 21 cows—mostly pregnant, and 20 calves of different ages) were injected subcutaneously each with 1 c.c. of the vaccine, Brew No. X/19-6-45. These injections were not followed by any appreciable local swelling or general disturbance. There has since been no report of any death due to anthrax in this herd. Previously, the disease broke out at least twice in the herd, on 2 September 1937 (one death) and on 25 May 1941 (three deaths). On both these occasions serum was used immediately and there were no further deaths.

In the second herd, a large commercial herd in Calcutta, a case of sudden death in a four-year old Sahiwal-Jersey first calver was reported on 15 September 1945. This heifer was said to have been all right till about 1 a.m. that morning when she was noticed to bellow a couple of times and, immediately afterwards, to fall down and die almost instantaneously. While making blood films from the carcase, which had already been opened, one of us (R.N.M.) noticed extensive subcutaneous haemorrhagic patches of different sizes over the chest, engorgement of superficial vessels, numerous petechiae on peritoneum and pleura, and sub-endocardial ecchymoses in the heart. The spleen was reported to have been normal, but the intestines had shown a swollen and haemorrhagic mucosa throughout. Examination of films made from the tar-like heart blood and also from the haemorrhagic subcutaneous tissue revealed numerous typical *B. anthracis*. The carcase was properly disposed of, necessary disinfection measures were immediately adopted, and the entire herd (strength shown below), down to one-day old calves, was vaccinated the same evening. The vaccine was of the same brew as that used at Midnapore.

Strength of the herd at the time of vaccination :

(*a*) Cattle :		
Bulls	10	
Cows in milk	223	(many pregnant)
Cows dry	145	(almost all pregnant)
Calves	243	(many sucklings)
Total	621	
(*b*) Buffaloes :		
In milk	20	(many pregnant)
Dry	14	(almost all pregnant)
Bull	1	
Calves	15	(mostly sucklings)
Total	50	

There was absolutely no post-vaccination disturbance in the general health of the animals, not even a reduction in the total milk yield of the herd. In most animals, however, small diffuse swellings developed over the seat of inoculation. These were neither distinctly hot nor painful and they disappeared in the course of one to three days.

No authentic history of anthrax occurring previously on this farm could be obtained, but it is possible such deaths might have occurred undetected.

On 9 February 1946, i.e., within five months after vaccination, cow No. 220 of this herd was noticed to be suddenly sick and restless. It died in a few minutes. The carcase was opened by the Farm Superintendent who found doubtful swelling of the spleen and some haemorrhagic patches on the intestinal mucosa in the region of colon and rectum. Films from heart blood revealed numerous *B. anthracis*. On 1 March 1946, the entire herd (650 head of cattle) was revaccinated with fresh vaccine, Brew No. 6/21-2-46. There have since been at least two deaths due to anthrax in this herd, the first on 24 August 1946, i.e., within six months after the March vaccination, and the

second on 12 January 1947. The diagnosis in both cases was confirmed on microscopical examination of films from peripheral blood of the carcases.

It is not desired to draw any conclusions from the small experience with these two herds. It tends to show, however, that the new Indian Veterinary Research Institute spore vaccine is perfectly safe for use in cattle and buffaloes in that, it does not give rise to much systemic disturbance or local swelling, and that the immunity following vaccination may not be expected to last for more than five months or so.

Inadvisability of large-scale annual vaccination

(*a*) *Economic considerations.* It has already been stated that the average recorded mortality due to anthrax amounts to only about 2·6 per cent of the total mortality due to contagious disease in bovines. While it is neither possible nor advisable to vaccinate every year the entire stock of the Province (over 30 millions), even in smaller areas such vaccinations are economically unjustified. Below are examined some data for two small Thana areas in Jhargram Sub-division of Midnapore district, localities which in the recent past have been affected with anthrax comparatively more frequently:

—	*Jhargram Thana*	*Nayagram Thana*
Total bovines (1945)	52,154	41,037
Annual mortality due to anthrax (five years' average)	under 5	under 12
This means one death due to anthrax for every	10,000 bovines	3,500 bovines
Cost of vaccine for every death due to anthrax	Rs. 2,500	Rs. 875

(*b*) *Technical considerations.* There are scarcely any rural areas, well-defined and limited in extent, such as villages or small groups of villages, where the disease may be said to be endemic so as to break out every year or so. Records obtained from the district veterinary assistant surgeons for the five-year period ending in 1944-45, and covering over 150 villages affected with anthrax, show that except two villages and one self-contained dairy herd, where the disease broke out twice, the outbreaks were not repeated in any village. This makes the task of undertaking large-scale controlled field trials of the vaccine as well as routine vaccination inadvisable. It must, however, be noted that, in view of the already stated difficulties in the way of correct diagnosis, the possibility of the disease becoming enzootic in some localities cannot altogether be ignored.

In the past, and even at present, the main suspected causes of widespread epizootics of anthrax have been lack of (*a*) prompt diagnosis and control of outbreaks and (*b*) proper hygienic measures, chiefly relating to disposal of carcases. For these reasons, it seems, the most profitable method of tackling anthrax in Bengal would be, one consisting of early diagnosis and institution of immediate combative measures, including judicious vaccinations and serum inoculations, as well as some well-organized attempts to ensure proper disposal of carcases. Prophylactic vaccinations on any large scale are not indicated, either on technical or on economic grounds, except in the case of valuable stock and, if required in future, in some badly affected localities.

Summary

1. Some aspects of anthrax in Bengal are described and certain relevant features discussed.

2. Anthrax was recognized to occur in Bengal at the inception of the Civil Veterinary Department in 1892.

3. Since then it has caused mortality chiefly in cattle, but to some extent also in other animals.

4. On several occasions the infection has spread to human beings.

5. Generally speaking, the extent of affection and mortality has been low and the outbreaks have been small in size.

6. There have been certain periods, of no obvious relationship, when epizootics of larger size were experienced.

7. The percentage of mortality recorded has varied annually from about 70 to about 85.

8. The clinical features have shown no substantial departure from the classical symptoms.

9. The disease has shown a definite seasonal prevalence in that roughly two-thirds of all outbreaks have occurred during the period April to July, i.e., in the summer and the first half of the rainy seasons.

10. The number of outbreaks occurring in different districts has generally been largely proportionate to their area and cattle population.

11. Certain districts, especially those in the combined Ganges-Brahmaputra deltaic area, have experienced comparatively heavy mortality.

12. Some experience with anti-anthrax serum inoculation and vaccination, including the new Indian Veterinary Research Institute spore vaccine, is described and the technical and economic aspects of such prophylactic measures are considered.

13. The new Indian Veterinary Research Institute spore vaccine has been found safe for use in all types of cattle and it probably confers immunity for about five months.

14. In view of the disease occurring in almost all parts of Bengal and in the absence of known endemic centres, it is considered that prompt diagnosis and suitable hygienic measures, combined with judicious vaccination, are likely to effect better control than large scale annual vaccinations, which are also uneconomical.

15. For valuable stock, however, prophylactic vaccination is recommended.

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OBSERVATIONS ON USE OF ANTHRAX SPORE VACCINE IN MADRAS

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(Received for publication on 29 July 1946)

THE use of anthrax spore vaccine in Madras was introduced in 1939. The results have been referred to in the reports of the Disease Investigation Officer of Madras [Viswanathan, 1937-45] and in the report of the Civil Veterinary Department of Madras for the year 1933-34. This article summarises some of the results observed.

Apart from vaccination, it is recognized that in order to achieve success in checking the disease, other measures required are, an early and correct diagnosis, as well as the proper disposal of carcases and proper cleaning of contaminated areas. While in most cases anthrax can be readily diagnozed by microscopical examination of blood smears taken immediately after death, the clinical symptoms and history are very helpful in making a tentative diagnosis. As to the use of anthrax serum for control, serum sometimes appears to be efficient in bovines and also in sheep in fresh centres of infection. In our hands, however, it has not proved effective in controlling outbreaks of 'subacute anthrax' in bovines, that is, outbreaks which tend to run a prolonged course. Nor has the serum been found helpful with sheep and goats, except in enzootic areas.

Spore Vaccine in Practice

The vaccine used was obtained from Mukteswar during 1939 to 1941 and from 1942 to date. During 1941-42 a thousand doses of vaccine prepared in Burma were used. The vaccine was given in 1·0 c.c. dose subcutaneously in the neck in bovines, sheep and goats; in elephants the injection was given into the subcutaneous tissue behind the elbow and was followed 20 days later by a second dose of 3·0 c.c. The vaccine has good keeping quality and under favourable storage conditions remains unchanged for some time, so that no difficulty was experienced in its transport and distribution in the field.

Unless assured as to the safety of the vaccine, small-scale preliminary tests were conducted on the particular species to be vaccinated. Thus in 1941-42 the Burma vaccine was tried during an outbreak of anthrax on six bovines, of which, four bullocks were given at the same time anthrax serum in dose of 20 c.c. There were no reactions. On another occasion spore vaccine from Mukteswar was used together with anthrax serum on five goats and 74 lambs. Serum was used for all the animals in dose of 20-40 c.c. the goats were given 1·0 c.c. of the vaccine and the sheep 0·5 c.c. The goats died of anthrax while the sheep were unaffected, showing that the vaccine was too severe for goats, at least in the dose given. This is in accord with the knowledge that it is more difficult to produce a suitable vaccine for goats than for sheep, though with the latter species also no small care has to be taken. As a result of the experiment just referred to, a milder vaccine and one suitable for goats as well as sheep is now supplied from Mukteswar. In company with Mr V. R. Rajagopalan, a safety test of spore vaccine was made on an elephant belonging to the Forest Department. In this case the vaccine was found to be safe when given in dosage of 1·0 c.c. followed 20 days later by 3·0 c.c.

In Table 1 are shown the species and number of animals treated with the spore vaccine, both at the time of outbreaks and in localities where no outbreak was prevailing. No animals could be left as unvaccinated controls in the particular herds or flocks being treated, though in the same villages some herds or flocks remain unvaccinated. In the case of sheep and goats some animals in free areas adjacent to the enzootic areas were first vaccinated as a trial. The results were encouraging in the sense that anthrax failed to appear in areas where anthrax carcases had been carelessly disposed of and where contamination was likely to have occurred. The elephants mostly belonged to the Forest Department and a few to private owners.

TABLE I

Anthrax vaccinations in Madras 1939 to 1946

	Bovines	Buffaloes	Sheep and goats	Elephants	Totals
Apart from outbreaks	61(1)	..	16,793(31)	120	16,974
During outbreaks	555(8)	108(3)	5,366(9)	..	6,029
TOTAL .	616	108	22,159	120	23,003

Figures in brackets indicate the number of places, where vaccination was done.

In cattle and buffaloes the reactions were negligible and consisted in a small proportion of cases of slight local swelling, slight fever and dullness. All these symptoms were transient. In elephants, only slight local swelling was seen. In sheep and goats, as noted by others e.g. Chadda [1939] symptoms due to the local irritant effect of the strong glycerol diluent were seen. Shortly after the injection, the animals started running round, jumping and bleating. Some lay down and rubbed their heads on the ground. Such symptoms caused alarm to the ryots but the signs of acute local irritation could be easily avoided by diluting the vaccine with two parts of normal saline solution prior to injection. With the first batch of vaccine from Mukteswar the reaction was very severe and 11·5 per cent of the injected animals (2,600 sheep and 88 goats) died, and some pregnant ewes aborted. With the milder batches subsequently supplied the reactions consisted only of slight local swelling and fever, though they were rather more severe in goats, a negligible percentage (below 1·0 per cent) died.

DURATION OF IMMUNITY

Mitchell [1930] in Burma states that from his experience of the immunization of animals against anthrax, the immunity lasts for about 12 to 16 months. It was found that sheep and goats left under natural conditions are unable to withstand three months later an inoculation of 1000 MLD of virulent anthrax culture. In this connection, 29 sheep and 25 goats, with six sheep to six goats as controls, were tested. That there is some residual immunity however is shown by the fact that after the test dose, the course of the disease in vaccinated animals is of two or three days, whereas in unvaccinated ones death occurs earlier. Also, after experimental inoculation, the mortality ratio as between vaccinated and non-vaccinated is 1 : 7·3, the numbers used being seven of the former and 51 of the latter. Since in practice sheep and goats do not contract anthrax within eight months after vaccination it would seem that they have a serviceable immunity at least for this period. In some of the enzootic areas annual vaccination of sheep and goats has completely stopped the disease. The vaccine indeed has become popular in the province. Ryots now understand the value of vaccination in minimising the losses from anthrax, whereas formerly they were often unwilling to permit vaccination since deaths from anthrax are usually few, at least at the beginning of outbreaks. Finally, a test with 1000 MLD of virulent culture applied by Mr Rajagopalan in Madras to an elephant 24 days after its second vaccination produced no reaction.

SUMMARY

Results obtained in Madras from the use of anthrax spore vaccine for cattle, buffaloes, sheep, goats and elephants are briefly described. Over twenty-two thousand sheep and goats and 120 elephants have been vaccinated during this work. The first vaccines tried were too virulent for

sheep and goats, especially for goats. The present vaccines, which are safe for these susceptible species, have been used as a preventive in areas hitherto free from anthrax and in enzootic areas, both in the presence and absence of outbreaks. Under these conditions outbreaks in cattle have been controlled and when used annually, vaccination has prevented the appearance of the disease in sheep and goats in areas where previously there was heavy mortality. There is reason to suppose that the period of effective immunity is not less than eight months.

ACKNOWLEDGEMENTS

Thanks are due to Messrs P. T. Saunders and T. J. Hurley, past and present Directors of Veterinary Service of the Province, to the staff of the Civil Veterinary Department of Madras and to members of the Mukteswar staff for their cooperation. We are obliged to Dr F. C. Minett for assistance in compiling this paper.

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ANAPHYLAXIS OR SERUM SICKNESS IN CATTLE

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(Received for publication on 19 September 1947)

FOR a number of years reports have occasionally been received at this Institute of accidents following the use in cattle of immune sera or vaccines. From the description received it is quite obvious that these accidents are anaphylactic in nature. Within a few minutes to several hours after injection of the product, any of the following symptoms may attract notice: generalized urticaria with itching, oedema of the head and perineal region, restlessness, excitement, tenderness at the site of injection, yawning, tremor, muscular weakness, dyspnoea, coughing. Deaths have occasionally been reported.

A few references have been found to the occurrence of anaphylaxis in cattle. Holmes [1913] refers to fatalities occurring in cattle after massive repeated injections of rinderpest blood and peritoneal washings. Since the material was homologous in kind and since symptoms were not immediate, with death as long as 2 to 11 days later, it is doubtful whether the illness so induced was true anaphylaxis. Jacob [1940] states that symptoms are often alarming but disappear within a few hours and death is rare.

Reichel [1939] quotes Hoskins to the effect that the period of incubation may be from 1 to 4 weeks. In some instances shock develops after what is alleged to be a first injection and even with homologous proteins. Such cases are perhaps to be explained by a specially high natural susceptibility, though it is not known in what proportion such cases occur. Even heated serum, homologous or heterologous, is capable of producing anaphylaxis. A possible but not very practical test for determining the sensitiveness of animals which are to get serum consists in an opthalmic test, in which a few drops of the serum diluted at 1 : 10 are placed on the conjunctiva. An intradermal test can be similarly employed.

In India, the occurrence of anaphylaxis or serum sickness in cattle is not widely known; no references for example have been found in the annual reports of the Disease Investigation Officers. On the other hand, colleagues with whom the matter has been discussed are aware of its existence. As concrete examples of anaphylaxis the following are worth mentioning. In the filed records of this Institute we find that in 1927 at the Hosur farm (Madras) 40 young cattle of Sindhi cross-bred, Ongole and Kangayam breeds were injected with anthrax serum and of these 18, aged 13-32 months, showed severe anaphylactic shock and one died. These animals had previously been given black-quarter aggressin and serum. In 1929 at the same farm 326 cattle were given rinderpest serum in the course of serum-simultaneous immunization, and as a result 51 showed symptoms of serum sickness and one died. The latter was a heifer of Ongole breed, aged three years. It developed symptoms within 10 minutes of being injected, viz. swellings in various parts of the body, distressed breathing and sub-normal temperature. On post-mortem examination haemorrhagic lesions were found in the submucous coats of the abomasum and intestine.

Even allowing for the facts that only a proportion of such accidents as those described are reported and that severe serum sickness must be comparatively rare cases, when they do occur, are naturally disturbing to field workers and deserve investigation. Some investigations indeed were carried on hill-bulls at this Institute by the late Mr Hugh Cooper and myself in 1930. These seemed to indicate that of biological products ordinarily used blackquarter agressin was the one most liable to be followed by anaphylaxis, provided the animal had previously received injections of immune sera. There were no deaths.

A fresh series of experiments was started at Mukteswar in 1944 in order to obtain further information on the liability of the local cattle to suffer anaphylaxis after being injected with various doses of immune serum at various times. Some work with blackquarter vaccine was also done. Finally some observations were made on methods of preventing attacks and the duration of the refractory state which immediately follows the reaction to an injected antigen.

EXPERIMENTAL

Kumauni hill-bulls were used as experimental animals, except in a few cases where cross-bred (Ayrshire-Sahiwal or Sindhi) calves were used. The products used were immune sera prepared against anthrax, blackquarter, bovine pasteurellosis and rinderpest. Since most, if not all of these products for veterinary use in India are prepared in buffaloes and since it was most likely that the trouble arose from the use of heterologous serum, some use was also made of normal buffalo serum. A few tests were also done with homologous serum. At the present time, aggressin is no longer manufactured in the Institute for prevention of blackquarter. Use was therefore made of the formalinized culture vaccine that is now issued; this is prepared in Noguchi medium in which is incorporated buffalo muscle extract and rabbit kidney.

The products were injected at intervals varying from 7 to 60 days, while a few regular reactors were tested after 13 months.

Incidence of anaphylaxis, symptoms

During the whole course of this work 260 hill-bulls and 12 cross-bred calves have been treated subcutaneously with various biological products, mostly immune serum made from buffaloes. It is highly improbable that these animals had been given any form of serum treatment previously. Of these 62 (22·8 per cent) showed symptoms of serum sickness and one animal died. Further details of this work are given below and the data are summarized in Table I. The incidence of reaction in the various groups was variable, e.g. in one batch of 124 bulls about 20·0 per cent reacted, in another group of 100 bulls 5·0 per cent reacted. The 62 cases could be divided into three groups according to their severity as slight, mild and severe reactors. Thus in 31 (11·4 per cent) reactions were slight, in 14 (5·1 per cent) they were mild, and in 17 (6·3 per cent) they were severe.

The symptoms produced varied considerably, from a few urticarial eruptions to severe shock. Many of the reactions would have been overlooked had not a thorough examination been made. In mild cases the only changes were urticarial swellings round the anus and on the perineum, slight swelling of the anus, and watery discharge from the eyes. Severe cases are indicated by pronounced oedematous swelling of the anus, perineum scrotum and sheath, of the head and neck or of the ears. There may also be severe urticarial eruptions over the body, marked swelling at the site of inoculation, restlessness, rapid and noisy dyspnoeic breathing, passage of dull-looking faeces which may be soft and blood-tinged. The animal does not feed and lies down. There is discharge from the eyes and nose, profuse salivation and sometimes muscular twitchings. The body temperature falls to 99°F. Tympanites is liable to appear and may cause death unless relieved. The appearance of urine is unchanged. The symptoms may develop as early as five minutes after subcutaneous inoculation and in many cases within 20 to 60 minutes. In mild cases the symptoms disappeared within 2-3 hours and in severe cases within 24 hours.

One bull, which died, developed acute tympanites before death. Noticeable changes were widespread cutaneous haemorrhages, oedema of the larynx, pharynx and base of the tongue with yellowish gelatinous exudate. There was some endocardial haemorrhage on the left ventricle, and congestion of various organs, e.g. the lungs.

Results obtained with repeated injections of various biological products

The results obtained with 19 hill-bulls and five cross-bred calves, which reacted at least once following the injections, are summarized in Table II. It will be seen that two subcutaneous injections of blackquarter serum at 20 or 40 days interval produced no symptoms. This in fact was the case with all the 62 reactors whatever the antigen. Their state of hypersensitiveness however is shown by the shock which follows the subcutaneous injection of rinderpest serum or blackquarter vaccine. Once the animals are sensitized in this way they are liable to be shocked by other immune sera, dosage being unimportant within the limits used, and the fact that they equally respond to serum from uninoculated buffaloes strongly suggests that it is the foreign serum protein which is responsible for the shock. At the same time serum from hill-bulls, i.e. homologous serum, is without effect. In these experiments the duration of the sensitive state was for at least 397 days.

TABLE I

Incidence of anaphylactic shock in Kumaunihill cattle and in cross-bred calves

No. of used cattle	Reacting	Symptoms		
		Slight	Mild	Severe
6 Hill bulls	4	1	—	3
10 Hill bulls	9	5	2	2
10 Hill bulls	6	4	2	—
124 Hill bulls	26	10	8	8
10 Hill bulls	7	5	—	2
100 Hill bulls	5	5	—	—
12 Calves (cross.bred)	5	1	2	2
TOTAL 272	62	31	14	17

TABLE II

Treatment of 19 hill-bulls and 5 cross-bred calves giving anaphylactic reactions

Serum injections Intervals (days) between injections :	1 B.Q.	2 B.Q. 40	3 R. 60	4 F. 40	5 AX. 40	6 B.Q. 40	7 Buff. 40	8 H. Bull. 45	9 Buff. 42	10 B.Q. 307
Hill-bull 150 .	15 c.c.—	15 c.c.—	15 c.c.+++	15 c.c.—	15 c.c.++	15 c.c.—	50 c.c.+++	25 c.c.—	20 c.c.+++	20 c.c.+++
,, 126 .	15 c.c.—	15 c.c.—	15 c.c.—	15 c.c.+	15 c.c.—	15 c.c.—	50 c.c.—	25 c.c.—	50 c.c.—	20 c.c.+
,, 251 .	15 c.c.—	50 c.c.—	50 c.c.+++	15 c.c.+	15 c.c.+	15 c.c.++	50 c.c.+++	25 c.c.—	20 c.c.+	20 c.c.+
,, 182 .	15 c.c.—	15 c.c.—	50c.c.—	15 c.c.+++	15 c.c.+++	15 c.c.++	50 c.c.+++	25 c.c.—	20 c.c.+++	20 c.c.+

Injections : Intervals (days) between injections :	1 B. Q. Serum	2 B. Q. Serum 20	3 B. Q. Vaccine 20	4 B. Q. Vaccine 20	5 Buff. Serum 55
Hill-bull 290	25 c.c.—	25 c.c.—	5 c.c.+	5 c.c.—	50 c.c.—
,, 268	25 c.c.—	25 c.c.—	5 c.c.+	5 c.c.—	50 c.c.—
,, 296	25 c.c.—	25 c.c.—	5 c.c.+++	5 c.c.—	50 c.c.—
,, 297	25 c.c.—	25 c.c.—	5 c.c.+	5 c.c.—	50 c.c.—
,, 259	25 c.c.—	25 c.c.—	5 c.c.++	5 c.c.—	50 c.c.—
,, 277	25 c.c.—	25 c.c.—	5 c.c.+	5 c.c.—	50 c.c.—
,, 291	25 c.c.—	25 c.c.—	5 c.c.++	5 c.c.—	50 c.c.—
,, 261	25 c.c.—	25 c.c.—	5 c.c.+	5 c.c.—	50 c.c.—
,, 281	25 c.c.—	25 c.c.—	5 c.c.+++	5 c.c.—	50 c.c.—

TABLE II—*contd.*

Injections: Intervals (days) between injections.	1 B. Q. Vaccine	2 B. Q. Vaccine 20	3 B. Q. Vaccine 20	4 B. Q. Serum 20	5 B. Q. Serum 20	6 Buff. Serum 35
Hill-bull 278 . .	5 c.c.—	5 c.c.—	5 c.c.+	25 c.c.—	25 c.c.—	50 c.c.—
„ 300 . .	5 c.c.—	5 c.c.—	5 c.c.++	25 c.c.—	25 c.c.—	50 c.c.—
„ 271 . .	5 c.c.—	5 c.c.—	5 c.c.+	25 c.c.—	25 c.c.—	50 c.c.—
„ 272 . .	5 c.c.—	5 c.c.—	5 c.c.++	25 c.c.—	25 c.c.—	50 c.c.—
„ 293 . .	5 c.c.—	5 c.c.—	5 c.c.+	25 c.c.—	25 c.c.—	50 c.c.—
„ 298 . .	5 c.c.—	5 c.c.—	5 c.c.+	25 c.c.—	25 c.c.—	50 c.c.—

Note.—Two other bulls in this series gave slight oedema of the eyelids 2 hours after injection 6

Serum injections*	1 B. Q.	2 B. Q.	3 B. Q.
Cross-bred calf 130	20 c.c.—	20 c.c.—	20 c.c.++
„ 132	20 c.c.—	20 c.c.—	20 c.c.+++
„ 393	20 c.c.—	20 c.c.—	20 c.c.++
„ 127	20 c.c.—	20 c.c.—	20 c.c.++
„ 123	20 c.c.—	20 c.c.—	20 c.c.+

*Intervals between injections:—40 days, with numbers 130, 132 and 393 and 20 days with numbers 127 and 123

Notes 1 *B.Q.=blackquarter serum
R=rinderpest serum
P=pasteurellosis serum (haemorrhagic septicaemia)
Ax=Anthrax
Buff. =healthy buffalo serum
H. bull =healthy hill-bull

2. — =no reaction
+ =very slight reaction, viz. few urticarial eruptions only
++ =mild reaction, viz., urticaria, oedema of anus, perineum and *C.* but no obvious systemic effects
+++ =relatively severe shock.

3. Table shows the number of inoculation, dose of serum or vaccine, and result

In most of the work the time interval between injections was fixed at 20 to 40 days. In order to see if a response would be obtained if the interval was shorter than 20 days, 10 hill-bulls were given three injections of 15 c.c. of blackquarter serum at seven-day intervals. As usual, the first two injections produced no response. After the third injection, seven of the 10 reacted mildly. Twenty days later, the 10 animals were again injected with 15 c.c. blackquarter serum, when two of the original reactors again responded this time severely.

Desensitization. It will be noted from Table II that animals are sometimes rendered insensitive by the one reaction so that later they do not respond to substantial amounts of antigen. This state of desensitation is well known to workers on the subject of anaphylaxis and it is said to be transient. Cattle for instance are said to remain insensitive to the shock for about three months, after which sensitivity gradually returns. From Table II however it can be seen that reacting animals sometimes responded to a fresh injection of antigen after 20 days and in many cases after 40 days.

An experiment was set up to throw more light on the duration of the desensitized state. Twelve hill-bulls which had reacted were selected and divided into three groups of four each. The animals in these groups were each reinjected twice with blackquarter serum: group I after 7 and 16 days, group II after 10 and 18 days, and group III after 14 and 20 days. The only further reactor was a bull of group I which gave mild reactions to both injections. This suggests that it is only the exceptional animal which will visibly respond to an antigen injected as early as a week after a previous reaction.

Experiments on prevention of shock

Three methods have been proposed: (1) The use of immune serum prepared from the same species of animal, (2) Dividing the amount of antigen into fractions, and giving one or more small doses and following up with the remainder of the amount after an hour or so. What may be called a modification of this method and (3) is to give the desensitizing dose of serum in diluted form, i.e. injection of reagents having an anti-histamine effect.

As to (1), the chief reason why buffalo serum is used in the immunization of cattle is apparently because, with the defibrinated or exalated blood of buffaloes, the red cells quickly sediment rendering the use of a centrifuge unnecessary. This is a very important consideration in serum institutes where large quantities of product have to be manufactured. The use of *Eos indicus* instead of *Eos bubalis* for serum production would introduce great difficulties in practice. As to (2), the method has been favourably reported upon and if found to be efficacious, should solve the problem. Thus Jacob [1940] has obtained useful results in this way. As to (3), a number of products have been tried and research in this field is continuing more particularly as regards prevention of atopic disorders such as asthma and hay fever. Thus, Dragstedt, Mills and Reed [1937] found that acute shock in sensitise dogs could be prevented by extract of adrenal cortex. Holmes [1913] and Reichel [1939] state that the simultaneous injection of a small dose of adrenalin, say 1·0 c.c. 1 : 1,000, will reduce the shock and the reagent may be mixed with the serum. Wolfram and Zwemer [1935] found cortin to be successful in sensitized guinea pigs.

In course of this work chief attention was paid to the second method, the principle of which is to desensitize the animal. Thus in December 1944, 124 hill-bulls were given 25 c.c. blackquarter serum followed 40 days later by a second similar dose. As expected, there were no reactions. Forty days later, the animals were divided into three groups: group I of 42 animals was given 25 c.c. blackquarter serum as a single dose and 10 reacted; group II of 41 animals was given 25 c.c. healthy buffalo serum as a single dose and seven reacted; in group III of 41 animals the dose of 25 c.c. blackquarter serum was divided: 14 animals were given 1·0 c.c. serum, 14 animals were given 2·0 c.c. serum and the remainder were given 5·0 c.c. serum, one hour later the remainder of the serum dose was injected. In this third group, nine animals reacted viz., two which had been started with the 1·0 c.c. dose, six which had been started with the 2·0 c.c. dose and one which had first been given the 5·0 c.c. dose. These 26 reactors and four non-reacting hill-bulls were then used for further experiments of the same kind. These animals were divided into two groups of 13 reactor and two non-reactors. After about 40 days, rest, group I was given 25 c.c. blackquarter serum in one dose and nine reacted (including one of the 'non-reacting' hill-bulls); in group II the dose of 25 c.c. blackquarter serum was divided into two parts, the first portion of 5·0 c.c. being followed an hour later by 20 c.c. In group II, eight animals reacted, viz., one severely after the 5 c.c. fraction and the rest after the remaining 20 c.c. fraction.

The experiment was continued with the 17 hill-bulls now left as reactors. These animals were divided into three groups of five, 6 and six bulls and tested 45 days later with blackquarter serum. The five animals in group I were given 25 c.c. serum as a single dose and all reacted. The animals of group 2 were given 5 c.c. serum followed two hours later by 20 c.c.; of these six animals, three reacted severely after the 5 c.c. fraction and one died, one reacted slightly after the 20 c.c. fraction, and two did not react. The animals of group III were given 1·0 c.c., 1·5 c.c. and 2·5 c.c. serum at two hourly intervals, followed two hours later by the 20 c.c. fraction; of these six animals, one reacted after the 1·5 c.c. fraction, one after the 2·5 c.c. fraction, two reacted after the final 20 c.c. fraction, and two did not react.

DISCUSSION

This work has again demonstrated that injections of a foreign antigen set up in cattle a state of hypersensitiveness, revealed by a definite train of symptoms of varying severity on reapplication of the antigen. Cattle vary considerably in their powers of response to the shocking agent, differing in this respect from guinea pigs and rabbits, on which most of the experimental work has hitherto been done. It has been shown that in the small hill cattle the sensitive state is not readily induced

and that more than one injection of the antigen at a suitable interval is required. How plains cattle compare in this respect cannot be said. It has also been shown that under the conditions of the experiments in hill cattle only about 20 per cent of the animals are sensitized sufficiently to respond visibly to a shock dose of antigen and that in only about 6 per cent are the symptoms of shock really severe. Under these circumstances it is doubtful whether in practice any special precautions aimed at preventing serum shock are likely to become popular. Nevertheless, the facts are worthy of note by field workers and it may be pointed out that biological products such as serum and vaccine should not be used on the same animals indiscriminately. Whether buffalo serum is more likely to produce shock than sera from other large animals or whether the incidence of anaphylaxis tends to be related to any particular season we do not know. The facts remain that buffalo serum excited shock in the present experiments and that most of the immune sera used for animals in India are prepared in buffaloes.

SUMMARY

1. An anaphylactic state can be induced in cattle by subcutaneous injections of immune sera prepared in buffaloes and by injections of blackquarter vaccine consisting of formalinized Noguchi medium culture. The symptoms of anaphylactic shock in cattle are described. About 20·0 per cent of hill cattle react with visible symptoms, in about 6·0 per cent the illness is severe, but less than 1·0 per cent die.

2. Not less than two sensitizing injections are required to produce a state in which visible symptoms will be elicited by further dosage of antigen. For a week or two after a shocking dose the animals are desensitized, while the sensitive state has been found to endure for upwards of a year.

3. Attempts at prevention by giving the shocking dose in small fractions at first, followed within an hour or so by the remainder were unsuccessful.

ACKNOWLEDGEMENTS

I am obliged to Dr F. C. Minett for suggestions and for assistance with the manuscript.

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CUTANEOUS ERUPTIONS IN RINDERPEST

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(Received for publication on 19 September 1947)

THE development of some form of eruption on the skin of cattle during the course of rinderpest has been noted by many observers. The phenomenon seems to have been recognized in India since time immemorial and, as in most other countries, the popular names given to rinderpest have usually been the same as for human pox. In Bengal particularly, it has received some attention in recent years, notably by MacGregor [1944] who, in collaboration with another officer [Mr M. B. Menon], made some preliminary observations and tests. The desirability of making further observations and critical tests to establish the association of the exanthema with rinderpest led to the problem becoming a part of the author's programme of work during 1943-1945. The work done and the information collected are described in the author's reports [Mohan, 1944, 1945] and are partly included in MacGregor's paper. In order to stimulate more interest in this aspect of rinderpest, a summary of these observations is given in this paper along with a résumé of relevant literature.

Literature

The cutaneous eruptions of rinderpest have been observed in almost all the European countries and in many parts of Africa. It is stated by Curasson [1921, 1932] that the cutaneous form of rinderpest had attracted the attention of the authors of the eighteenth century and that the relationship between the exanthema and the infection had been recognized as early as 1712 by Ramazzini. In those days, rinderpest is reported to have been classed among the eruptive fevers, as it tended to simulate serious forms of vaccinia [peste varioleuse de Vicq-d'Azyr] and, according to Gerlach [1873], Ramazzini went so far as to designate rinderpest as 'pockenseuche', *i.e.*,'pock disease'. The development of the eruptions as a characteristic feature of rinderpest was recognized by later writers also, but once the specific relationship was established no further importance was attached to them, so that the subject was gradually forgotten and was mentioned only exceptionally. The eruptions were repeatedly noted during the great wars of the nineteenth century and were described by Gerlach (*loc. cit.*), Peuch [1892], Nocard and Leclainche [1903], and other European writers. Gerlach's notes were based on observations made chiefly in Holland and Germany, but also in Hungary and England. Gärtner [1920] found them to be a common feature in East Africa. Hutyra and Marek [1916] described them from Roumania and noted their frequent occurrence in cattle of the steppes of Russia, where considerable importance has been attached to the phenomenon. Curasson (*loc. cit.*) and his colleagues working in West Africa during the epizootics of 1916-1919 found the eruptions a more or less constant feature in Sudan and other parts. Croveri [1921] found them very frequent in Italian Somaliland, Carlier [1920] in Belgian Congo, and Pecaud [1924] in Chad. Malfrey [1927] and Schein [cited by Curasson, 1932] have discussed the phenomenon and have sought explanation in climatic influences.

Angeloff [1918], on the other hand, noted the eruptions in only two cases out of several hundred during the 1912-13 Balkan War, and Malfrey [cited by Curasson] observed them in only exceptional cases in Nigeria during the 1916-1919 epizootics. Vrijburgh [cited by Hutyra, Marek and Manninger, 1938] and Melanidi and Stylianopoulo [1927] never observed any cutaneous lesions in West Indies and Greece, respectively.

In India, MacGregor (*loc. cit.*) seems to have been the only modern writer to draw attention to the appearance of these eruptions. The Indian Cattle Plague Commission's report [1871] contains several interesting references to the subject. A few selected extracts are given below.

Page 59. (A letter from Hiram Farrell, Veterinary Surgeon, His Majesty's Bengal Army, to Baboo Hem Chander Kerr, Deputy Magistrate, Diamond Harbour, District 24-Parganas, Bengal.) '.....body is covered with small vesicles resembling pimples (which later) become ulcerated, dry and covered with scabs.....I have invariably found that the greater the amount of eruption the more favourable was the case'.

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Page 75. (Evidence of T. L. Taylor, Superintendent, Municipal Gaokhana, Calcutta). '..... eruption in some cases occurring generally on the loins, fore shoulders, but not on the belly. The eruption is dry, and in passing the hand over the skin it feels like hard pimples. In some cases I have altogether failed to detect eruptions or pimples of any kind in any place..... Cattle showing an eruption generally die more rapidly than those which have none.

Page 84. (Evidence of Brojo Kishore Ghose, 25, a resident of Korojolee near Diamond Harbour, District 24-Parganas.) Generally speaking, in sporadic form, or in those cases occurring at a time when the disease has not assumed an epizootic form, very often the cattle affected do not entirely lose their appetite and do not, as a rule, purge, but in such cases eruptions on the skin are almost sure to make their appearance. The animal generally gets well. When purging takes place and there is no eruption it is nearly always fatal.

Page 97. (Information given by three residents of Dowlat Dyar, District Nadia, Bengal.) .....in some cases an eruption appears on or about the seventh day (one or two days after diarrhoea), at first in the form of spots, and the skin peals off.

Page 137. (A notice of bussanto by Mr Lamb, Civil Surgeon, Dacca, in a letter dated 1836 to the Superintending Surgeon of the Circle). On the fifth day (of the fever) the eruption appears about the udder, sometimes only a few pustules, at other times, they are numerous and confluent; but the result does not seem much dependent on the eruption. Whether the pustules are numerous or rare, the disease is almost equally fatal..... When the hair is rubbed off after the pustules have appeared on the udder, the skin is found covered with sores; many die before the eruption makes its appearance.

Page 196. (Extracts from records of Medical Department, Lohardugga District, Chhota Nagpur. A letter from W. Dunbar, Assistant Surgeon, Dorunda, dated March 1836.).....towards the close of the disease in such cases (*i.e.*, which recover) an eruption breaks out over the skin, of a reddish dusky colour, each spot or pustule being about the size of a split pea. The eruption, as the animal gets well, generally disappears.

Similar evidence was available concerning the Central Provinces, Sind and South India (Canara).

Frequency of appearance

From the foregoing résumé of literature it will be seen that while some observers found the eruptions to be rare or uncommon, there are many others, notably Curasson, who regard them as a more or less constant feature of the disease appearing almost as commonly as other symptoms like mouth lesions and diarrhoea. Gärtner (*loc. cit.*) observed generalized exanthema in 60 to 80 per cent of affected animals. From the fact that skin eruptions are seldom reported from most parts of India, where epizootics of different dimensions break out practically every year, the experience of this country would place the frequency as very low indeed. At any rate, the eruptions, if they develop, do not appear generally to do so in sufficient numbers and severity to attract attention. In Bengal, on the other hand, they seem to have been observed comparatively frequently, though here again there is some difference of opinion with regard to their frequency. The experience gained in the past few years shows that they are not a common feature, as most of the animals examined during a number of outbreaks of rinderpest failed to show any eruptions. During the period 1941-1945, the eruptions were noticed in nearly twenty outbreaks, out of several hundreds, in different parts of the Province.

It must, however, be recognized that, on account of their minute size in the earlier stages and the more or less hairy coat of cattle, the eruptions are liable to be missed in most cases, unless specially looked for particularly when they appear in small numbers only. Moreover, a large proportion of the animals affected with rinderpest in India die before any skin eruptions are detected fully.

Description and course of the eruptions

Among the western writers, the eruptions have been well described by both Gerlach and Curasson. As these descriptions are in German and French and not available to most field workers in India, some relevant translated extracts are given below.

Gerlach (*1873*). The skin is affected principally in the same way as the mucous membrane, but not so constantly, and generally in a few places only. If the lesions are few in number they are liable to be missed. It is always the fine, especially the unpigmented, skin on which the exanthema appears, the favourite sites being the udder, especially the base of the teats, and the scrotum. Less frequently, it may be seen on the nasal alae and vulvar labiae, perineum, inside of the thighs, on the head and neck ; very seldom, it gets generalized. As with lesions on the mucosa, the course of the skin eruptions varies with the severity of the initial lesion. The mildest lesions lead to rich desquamation and yellowish brown crusts cover the areas. The next higher grade shows itself by hyperaemia, diffuse erysipelatous reddening, much peeling off, transudation, moistening and greasing of the affected spots, and finally formation of thin scabs. The severest lesions consist of intense reddening, destruction of epidermis and formation of thick crusts which are dirty yellowish brown, often many layers thick, and firmly set over the dark red corium. The lesions of milder degrees occur in diffuse form over large areas, particularly on the neck and inside of the thighs. Those of the severest degree are generally localized to the udder, scrotum and perineum. Vesicles and pustules do not form, but the eruption on the udder is reminiscent of pox.

Curasson (*1921, 1932*). The cutaneous manifestations are a constant symptom of rinderpest. They appear in the second stage of the attack simultaneously with conjunctivitis, stomatitis and diarrhoea, but in view of their small size and discrete nature they are generally noticed much later than the concomitant lesions. The red, round and more or less discrete or confluent spots (macules) occur on different parts of the body, more commonly where the skin is thin and the hair fine, less frequently on the upper parts of the body and the extremities, exceptionally below the hock or the knee. On these spots, the hair stands erect and, if the lesions are isolated, the small bunches of hair appear as in miliary ringworm. As they porgress, the macules rapidly become papules, and later vesico-pustules ; at this time they generally attract attention. In two or three days, transudation starts, the pustules burst causing matting of the hair, and the picture varies according to the number and disposition of the lesions. When localized to areas of fine skin like the udder, and when they dry up, the crusts and the matted hair fall off leaving small gaps in the coat. The hair on these spots reappears slowly and the small ' tufts grow against the grain '. If, on the other hand, the pustules involve more or less large plaques, the skin gets raised by fluid collected in layers in the thickness of the dermis, but following transudation on the surface it subsides, when it may crepitate. Later, the corresponding pieces of epidermis fall off and the dermis which is laid bare is in a state of congestion, which disappears slowly, and is like a raw surface left after strong vesication or blistering. On these parts, the hair grows slowly, is always sparse, and its colour and direction are different from the surrounding coat. Such lesions are commonly encountered on the back, loins and croup. Very rarely, the eruptions become generalized, covering the whole body, and resulting in the formation of large plaques. When the scabs from these areas fall off, an intensely congested, naked, and badly damaged skin is left behind, as in severe vesication. The deeper papillary layers of dermis are destroyed and the hair does not grow again ; veritable cicatrices remain.

Eruptions as seen in Bengal

The initial lesions, when noticed, are said to be in the form of small, discrete, reddish spots, ' like mosquito-bites ', appearing about the same time as the mouth lesions, diarrhoea, etc. These develop and turn into pustules about the size of a split pea in diameter, and some thick sero-purulent matter is discharged which causes the hair to mat in isolated bunches. The scabs which form stick to the body of the animal for about five to ten days, sometimes even longer, and, when the dried up crusts fall off, small hairless islets are left behind. New hair grow up after some time on these spots and in four to six weeks attains the level of the surrounding coat from which it may generally be distinguished by appreciable differences in colour and direction. In many cases, the pustules are ill developed, especially those on the back and chest, and there is little transudation, so that the crusts stick closely to the underlying skin and do not fall off until some weeks after their formation. The commonest sites of the cutaneous eruptions seem to be the region of the shoulder and the adjoining areas on the chest, back, neck and dewlap. They have, however, been noticed on almost

any part of the skin extending dorsally from the muffle to the anal region, laterally from the cheek to the back of the thighs, and ventrally from the jowl to the perineum in both sexes, including both sides of the upper parts of the legs. They make their appearance in a single crop, but their numbers may vary from a few scattered lesions to several hundreds, with a tendency to form groups of closely situated eruptions on some areas, when they may coalesce and on being shed leave behind bigger patches of raw skin. The severest types of lesions described by the European observers are seldom seen in Bengal, except occasionally in buffaloes in which many large pieces of epidermis may come off.

Association of eruptions with rinderpest

Certain observers who appear to have had little experience of the true rinderpest exanthema have doubted the development of any specific eruptions in this disease and have even suggested that these may only be the result of some non-specific factors like tick-bites, impetigo, etc. While the possibility of such non-specific lesions being sometimes mistaken for true rinderpest exanthema, especially by those workers who have had no previous experience of the latter, cannot be denied, the eruptions which usually develop during the course of rinderpest can generally be recognized without much difficulty. Though the experience of workers in India and abroad left little doubt of the true relationship between the infection and the eruptions, it was considered desirable to provide some positive evidence to support this.

In most of the outbreaks investigated ample clinical evidence diagnostic of rinderpest was available. Because of the delay which usually occurred in reporting and contacting the scene of the outbreak, blood transmission could not be undertaken. Attempts to transmit the infection with material consisting of scabs collected from affected animals in the 'field' and transported to the laboratories in Calcutta had to be resorted to, so that usually many days elapsed before the material could be put to test. In all, about a dozen tests were done, with scab suspensions in 0·5 per cent saline inoculated sub-cutaneously into goats, calves and buffaloes, and also applied by scarification on the external ear. In the majority of cases, the results were negative, but in at least two instances the disease was definitely reproduced. In one of the positive cases, the scabs had remained at room temperature for nearly three weeks before test.

Related clinical and epizootiological aspects

The appearance of the cutaneous eruptions on animals affected with rinderpest has generally been a welcome feature in view of the popular belief that such attacks are comparatively mild and more commonly followed by recovery. A similar notion regarding many human diseases is also prevalent in many parts of the world. Actual scientific data in support of this contention have never been forthcoming, but the experience of most observers shows that the eruptions, though often seen in severely affected animals, are usually a feature of milder attacks. Again, they have been more commonly observed in outbreaks of moderate severity than in those accompanied by heavy mortality. The experience in Bengal has been identical and the eruptions have been much more commonly seen in isolated and mild outbreaks and towards the fag-end of bigger epizootics than in the midst of severe and widespread outbreaks. In view, however, of the nature and course of the eruptions, as already stated, their comparative infrequency in animals dying of acute attacks of rinderpest may be more apparent than real.

Below are given (Table I) some relevant particulars of two outbreaks in which cutaneous eruptions developed. These may serve to illustrate the usual course of events in such cases.

TABLE I

Results of two outbreaks in which cutaneous eruptions developed

	Outbreak I	Outbreak II
Place affected	District Jalpaigori (north-west Bengal).	District Noakhali (south-east Bengal)
Date of outbreak	July-August, 1941	September-October, 1944
Total number of animals affected	14	17
Number of animals showing cutaneous eruptions	5 (36 per cent)	7 (41 per cent)
Total mortality	10 (71 per cent)	10 (59 per cent)
Mortality in 'cutaneous' cases	3 (60 per cent)	2 (29 per cent) Both deaths were delayed
Mortality in 'non-cutaneous' cases	7 (78 per cent)	8 (80 per cent) Four deaths occurred within 4 days, the remaining in 6 to 10 days after onset of attack.

Both in Bengal and elsewhere, the eruptions have been reported more frequently in certain years (or series of years) than in others, but the exact significance of this variation is not understood. The identical nature of the popular labels given to rinderpest in different parts of the world (though very often the same labels have been used to designate many other diseases), and the fact that the cutaneous eruptions had attracted the particular attention of the earlier writers, are considered, perhaps correctly, by some modern observers to signify that originally rinderpest was more a skin affection, like vaccinia, than a generalized organic infection, as commonly seen now-a-days. It seems possible that rinderpest virus is gradually becoming more organotropic and less dermotropic. Even to-day, some observers in Bengal would go so far as to assert the occurrence of non-fatal outbreaks of more or less pure 'cutaneous' form of the disease, unaccompanied by much systemic disturbance beyond a little rise in temperature. There have been at least two such instances in this Province in the recent past, but the opinions of experienced officers who visited the affected place were sharply divided about the authenticity of these outbreaks. Unfortunately, no conclusive proof by way of biological tests was secured and the disease could not be reproduced by inoculation of scab material, so that it is not possible to make any decisive statement about the outbreaks. The eruptions in most cases were in a very much exaggerated form and, on the whole, were very unlike those usually seen in rinderpest. Macgregor (*loc. cit.*) has included photographs of these eruptions.

Some workers have tried to seek some sort of relationship between the eruptions and the prevailing weather conditions. Thus, Malfrey [1927] considers them to be more frequent in the rainy season, especially on those animals which are exposed to showers of rain. According to him, the eruption is a reaction of the hyperaemic skin to the rain, while Curasson quotes Schem as regarding

it a reaction to the Sun rays. The recent outbreaks of 'cutaneous' rinderpest in Bengal were not confined to any particular part of the year, but the majority of them occurred during the rainy season and in the autumn (*i.e.*, April to November), when both atmospheric temperature and humidity are high. There has, however, been no regional localization, almost all parts of the Province having reported eruptions at different times. The annual rainfall in the different districts varies from about 55 to about 130 inches.

As a rarely, or even frequently, developing symptom of rinderpest, the cutaneous eruptions are perhaps of no more than a little clinical interest to-day. There is no doubt that the scabs tend to stick to the affected animals for several days and thus may play some part in disseminating the infection, in view of the fact that the rinderpest virus may persist in them in a viable condition. Their rôle in the causation of the so-called 'spontaneous' outbreaks of the disease [MacGregor, *loc. cit.*], however, seems to have been unduly exaggerated, like that of the hides.

Summary

1. A consolidated summary of some observations recently made in Bengal concerning the appearance of cutaneous eruptions in cattle affected with rinderpest is given along with a résumé of literature on the subject.

2. The nature and course of these eruptions are described and some related clinical and epizootiological aspects are discussed.

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VERMINOUS PNEUMONIA IN ANIMALS IN BENGAL

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(Received for publication on 24 September 1947)

Experience gained during the last decade or so, has taught us, that worms are an important cause of pneumonia in domestic animals in India. The lung-worms (Metastrongyles) have been encountered frequently in different parts of the country and in many places they have been found to cause serious losses, particularly among the goats. The observations made in Bengal show that conditions in this Province are more or less the same as in other parts of India. These observations, especially those made during the last five years, are briefly described below.

Verminous pneumonia in cattle

Dictyocaulus viviparus is the universal parasite of the bovine lung and is known to cause much damage and deaths in calves. It has also been held responsible, especially in Europe, for serious occasional outbreaks of pneumonia in adult cattle. This worm has been frequently encountered in all parts of India, but very little information is available regarding the extent of damage done by it. The following notes concern the position with regard to this worm in Bengal.

Lung-worms in calves. On several occasions these worms were found in the bronchi of slaughtered calves which were discontinued from 'pox lymph' production. They were also encountered on a few occasions during post-mortem examination of some dairy calves. Generally speaking, the infestation was light, but, while macroscopic lesions in the lungs were seldom detected in the slaughter-house, a varying extent of consolidation, at least partly due to the presence of the worms, was often noticed in the lungs of dairy calves. Deaths among calves in dairy herds and outside have not been systematically investigated in Bengal, so that it is not possible to guess the extent of damage which lung-worms may cause. Although there are no known instances of deaths due to verminous pneumonia in calves in this Province, there is reason to suspect that these worms play their rôle at least in undermining the health of the calves.

Lung-worms in adult cattle. Lung-worms were seldom encountered in adult cattle in the slaughter-house and when found, they were generally present in small numbers. Examination of a few carcases of cattle dying of different diseases also did not reveal any.

In 1942-43 [Mohan, 1943], however, there occurred a serious outbreak of pneumonia which killed many cows of a big commercial dairy herd in Calcutta. Investigations conducted showed that the primary cause was *Dictyocaulus viviparus*. In view of its interesting nature and the fact that such occurrence has not been previously recorded in India, it is described somewhat fully below.

The outbreak was the first of its kind that ever occurred on this farm. There was reason to suspect that the disease was imported with a batch of cows purchased from the Punjab in May 1942. By October 1942, several cows were noticed affected and six cows, all belonging to the May lot, died of pneumonia. In the subsequent months, a gradually increasing number of animals were noticed to be coughing and 32 more cows died out of a total of about 200. Besides, some 80 cows, majority affected and many in advanced stages, were sold off. It was not, however, until February 1943, *i.e.*, several months after the first deaths, that the matter was reported to the Veterinary Department, so that the investigation and control could not be undertaken earlier.

On account of this great delay in the reporting of the outbreak and the absence of reliable records concerning the earlier cases, it is not possible to state when actually the disease started and when the first death due to pneumonia occurred. The description given in this note is, therefore, confined to such observations which are considered more or less authentic. In the beginning, the number of cattle showing prominent clinical evidence of the disease was small and the first few deaths occurred at intervals of several days and weeks. Later, however, especially towards the end of the prolonged course of the outbreak in the herd, the number of affected animals increased considerably and deaths occurred at much smaller intervals.

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† Working on a scheme partly financed by the Indian Council of Agricultural Research.

Clinically, the disease was ushered in by an occasional dry and husky cough which generally passed unnoticed till it gradually became more frequent. As the disease progressed, there was some loss of condition accompanied by depressed appetite and irregular bowels. Later on, there was some discharge from the nose, usually in the form of masses of mucus expelled during paroxysms of coughing. In fatal cases, extensive involvement of the lungs became apparent and all the symptoms of a true pneumonia developed, marked dyspnoea being prominent. In animals thus affected there was a more or less complete loss of appetite, the rectal temperatures rose high, and many developed diarrhoea. Once true pneumonia set in, death followed in a fortnight or so, though occasional cases lingered somewhat longer. Recoveries were common only in those cases which did not show signs of the lungs being involved to any considerable extent.

On post-mortem examination of a few carcases, the organs found mainly affected were the lungs. These commonly showed extensive consolidation accompanied by varying degrees of thickening and distension of the inter-lobular septa in parts. In some cases, there were either small discrete foci or bigger areas of suppuration. The basic morbid change uniformly present was an interstitial *cum* catarrhal pneumonia, typical of verminous pneumonia. There was no evidence of pleuritis. The trachea, and especially the bronchi, showed inflammatory and catarrhal changes and they always contained the worms. *Dictyocaulus viviparus*, generally in large numbers.

On microscopical examination, numerous typical metastrongyle eggs and larvae were seen in fresh cover-glass preparations of lung exudate. Stained films of the exudate revealed a variety of organisms among which chiefly two types predominated. In films made from the more or less uniformly consolidated non-suppurating parts, the predominant organism was a Gram-negative coccobacillus having some tendency towards 'bipolar' staining, while in those from suppurating parts it was a Gram-positive diphtheroid. Due to lack of suitable facilities no cultural work could be undertaken, so that it is not possible to state the exact identity of these organisms. It may be mentioned here, that in studies undertaken earlier by the author at the Indian Veterinary Research Institute, Mukteswar, on the bacteriological flora of normal and pathological lungs of ruminants, the two pathogenic organisms most commonly isolated were *Corynebacterium pyogenes* and those known as 'haemolytic cocco-bacilli'. This is similar to the findings of other workers abroad [Bosworth and Lovell, 1944]. In view of this, it seems likely that these two were the organisms secondarily concerned in this outbreak also.

Stained films of peripheral blood of affected animals and peripheral and heart blood of carcases were uniformly negative. Histological examination confirmed the macroscopic and the microscopic findings. The basic picture was that of verminous pneumonia, but at many places this was more or less completely masked by the superimposed bacterial pneumonia.

The results of these investigations left little doubt with regard to the correct nature of the diagnosis. It was nevertheless considered desirable to examine the possibility of co-existing contagious bovine pleuropneumonia. This was ruled out by the complete lack of reaction in two bulls inoculated subcutaneously with lung exudate from a somewhat suspicious case.

The affection did not spread to the suckling calves and the few deaths which occurred among them in this period were due to other causes.

Lung-worms in sheep

Dictyocaulus filaria and the pulmonary changes associated with it, *i.e.*, typical verminous pneumonia, were often encountered in plains sheep in Calcutta slaughter-houses. Their occurrence in hill sheep in Darjeeling slaughter-house was noted to be more common and the infested lungs generally showed patchy consolidation, especially in the apical and cardiac lobes [Mohan, 1945]. Balwant Singh [1937] encountered *Varestrongylus pneumonicus* also in a few hill sheep.

Bengal is not a sheep-raising country and the occurrence of fatal outbreaks of pneumonia in this species is not known.

LUNG-WORMS IN GOATS

Mortality due to verminous pneumonia in goats in Bengal was first investigated and reported by Balwant Singh [1938, 1939, 1940], who found almost all flocks in Darjeeling district affected. The extent of affection and mortality in these flocks was very variable, but there were instances of whole flocks having been lost.

The lung-worm most commonly encountered was *Varestrongylus pneumonicus*. In some cases, *Dictyocaulus filaria* was also present. The infestation was generally heavy and the lungs showed evidence of severe broncho-pneumonia accompanied by much consolidation, especially of the anterior lobes. Scattered patches of pure verminous pneumonia were a constant feature. The histological features were, an interstitial *cum* broncho-pneumonia, marked by the presence of worms, eggs and larvae in sections (as in lung exudate), and in some cases purulent foci were seen. The disease could not be reproduced by experimental inoculations with lung exudate and a *Pasteurella* type of organism, once isolated from an affected lung, proved non-pathogenic for bovines.

The main clinical symptoms were, gradually increasing loss of condition and a persistent cough which was dry in the earlier stages but moist and accompanied by a muco-purulent nasal discharge in advanced cases. The rectal temperatures often rose to 106°F and some goats developed diarrhoea. The disease was found more prevalent during the winter months.

LUNG-WORMS IN SWINE

The occurrence of *Metastrongylus elongatus* in pigs in Bengal has also been noted [Balwant Singh, 1937; Mohan, 1945]. Balwant Singh found these worms in nearly 50 per cent of slaughtered pigs. Mohan has recorded serious mortality caused by pneumonia in piglings of a big commercial farm in Darjeeling district. The two worms which seemed to be concerned in mortality were *Matastrongylus elongatus* and *Ascaris lumbricoides*. In most cases in which the lungs were extensively consolidated, the bronchi were found packed with dense masses of *M. elongatus*, and there was little doubt of their fatal rôle. In those few cases in which the lung-worms were few or absent, the round worms, which were present in very large numbers in the intestines of such animals, must have contributed considerably towards undermining the general health of the piglings and, in view of their life-cycle, may also have had some part in causing pneumonia. Microscopical and histopathological examinations revealed numerous metastrongyle eggs and larvae and small Gram-negative cocco-bacillary organisms, the latter generally present in the form of large focal agglomerations.

The recording of *Metastrongylus elongatus* in this country is of special interest. Baylis and Daubney [1923] recorded only fragmentary material of this species from the bronchi of a pig in the collection of the Zoological Survey of India. In view of this, Baylis [1936] says, 'it is uncertain whether the specimens were of Indian origin', though Bhalerao [1935] has included the worm in his catalogue of the helminth parasites of domestic animals in India. The present record should leave no doubt of the widespread occurrence of this parasite in India, as it has been repeatedly encountered in the hills and the plains, and in pigs bred locally, in Orissa, and in the United Provinces.

SUMMARY

1. The occurrence of lung-worms and verminous broncho-pneumonia in domestic animals in Bengal is recorded.

2. Fatal outbreaks of pneumonia, primarily verminous, in adult cattle, goats and piglings are described.

3. The worms incriminated are *Dictyocaulus viviparus* in cattle, *Dictyocaulus filaria* in sheep, *Varestrongylus pneumonicus* in goats, and *Metastrongylus elongatus* in pigs.

The occasional occurrence of *Dictyocaulus filaria* in goats and *Varestrongylus pneumonicus* in sheep is noted.

4. *Metastrongylus elongatus* may be accepted as a well established parasite of pigs in India.

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DENTITION IN INDIAN CATTLE

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(Received for publication on 8 October 1947)

Indian farmers, veterinarians and others when ageing cattle, have not employed the western standards but have modified them in accordance with experience. There exist no easily available recorded data concerning what these modifications should be, and it was, therefore, decided with the help of government farm staff to make observations anew and to record them forthwith. What follows is the result of that work.

The permanent dentition of the ox consists of the following teeth $\frac{0033}{4033}$. The temporary or milk dentition is $\frac{0030}{4030}$.

Observation has been made of the teeth of individuals in various age groups in most of the important breeds of the country, viz. Hariana (from Bengal, Hissar, Quaderabad and the United Provinces districts), Sindhi, Sahiwal (from the Punjab and Delhi), Hallikars, Ongoles, Kangyam, Amritmahal (from Bombay and Mysore). Gir, Dangi, Kherrigahr and Ponwar. The number of animals examined, in various ages has varied in each case according to the facilities or the number of the animals available at the time of the examination, but on account of the similarity in the samples, statistically, it is considered that the number of animals examined are enough and can be taken to represent the population of that breed as a whole. Teeth were examined at one month (in some cases), three months, six months, one year, one and a half years, two years (in some cases), two and a half years, three years, four years, four and a half years (in some cases), five years and six years. The dentition observed at each of these age periods probably represents the dentition at the age specified plus or minus one month because of possible inaccuracy in the dates of birth or because of the fact that in some farms the animals are aged according to the quarter of the year in which they are born.

The following Table I illustrates the order of eruption of the teeth of individuals of the Hariana breed.

Table 1

Eruption of the various teeth in Hariana Cattle

Time of eruption	Incisors 1	2	3	4	Molars 1	2	3	4	5	6
At 1 month	†	†	†	†	†	†	†			
„ 3 months	†	†	†	†	†	†	†			
„ 6 „	†	†	†	†	†	†	†	†		
„ 9 „	†	†	†	†	†	†	†	*		
„ 1 year	†	†	†	†	†	†	†	*		
„ 1½ years	†	†	†	†	†	†	†	*	*	
„ 2 „	*	†	†	†	*	*	†	*	*	*
„ 2½ „	*	†	†	†	*	*	*	*	*	*
„ 3 „	*	*	†	†	*	*	*	*	*	*
„ 4 „	*	*	*	†	*	*	*	*	*	*
„ 5 „	*	*	*	*	*	*	*	*	*	*
„ 6 „	*	*	*	*	*	*	*	*	*	*

* Permanent dentition.
† Temporary dentition.

All temporary incisors and the first, second and third temporary molars are present at one month. The fourth permanent molar erupts in 33 per cent of the animals at the age of six months, and is completely up in all the animals by nine months. At one and a half years the fifth permanent molar is up in 50 per cent of the animals, while at two years all the permanent molars except the third are through and rising. At two and a half years they are well up. The first pair of incisors are just rising in 50 per cent of the animals at two years, while at two and half years they are well up. By the third year the first and second pairs of incisors are up, and the set of permanent molars is complete. In the fourth year the third pair of incisors is up. The third pair of incisors are up soon after one month of the second pair. The corner pairs appear sometimes in the fourth year but are only well up at four and half a years. The permanent dentition is complete between four and a half to five years, and all the four pairs of incisors have been brought into wear. In the Hariana cattle examined in Bengal, dentition is slightly later. The fourth permanent molar is seen in all cases at about nine months, and the first pair of incisors in all cases is erupted at two and a half years. In all other breeds the same type of dentition is observed as in the Harianas in Bengal, *i.e.* the first pair of incisors is up at two and a half years, and the permanent dentition is complete at five years. Differences are observed in the case of Amritmahal, in some of which full mouth is obtained a bit later.

In Ponwars, the fourth permanent molar erupts at three months, while the fourth and fifth both are well up at six months. At nine months the sixth permanent molars are up. The first pair of incisors erupts at one and a half years, the second at three years and the third and fourth at four years. Some observations were made in the case of Terai buffalo, in which dentition closely corresponds to that of Ponwar. In Sahiwals, except that the fourth permanent molar is up earlier, *i.e.* it starts erupting at three months, and is up at six months, the usual trend of dentition is the same as in the Hariana and the same is the case with the Sindhis. The Sahiwal herd at the Indian Agricultural Research Institute, which is kept under an intensive system of management, did not show any significant variation in dentition from other Sahiwals. The central permanent incisors are up at two years, corners start coming up at four years one month or so in number of cases and the mouth is complete at four and a half to five years. Table I may therefore be taken to represent the dentition more or less for all the breeds in India except for minor differences here and there and for the fact that in most of them (except Harianas and Sahiwals in the Punjab) the first pair of incisors is up at two and a half years. All the animals examined were from the various Government farms, but as no intensive feeding on western lines is practised (except where stated) in any of these farms, it is likely that the dentition in village cattle will be very much the same. There was no difference however, in dentition, between the males and females.

Buffaloes

The dentition in buffaloes is more or less the same as that of Indian cattle. In their case, the central as well as the medial temporary incisors are up even at birth. At one month the three pairs and at three months all the four pairs are up. At three months the corners are just erupting and are overlapped at laterals. This overlapping of the corner is noticeable at six months but none is observed at one year when wearing surfaces are also more extended. At two years, corners have a characteristic depression on the wearing surface and the centrals are more spaced and have a necked appearance. At two and a half years, the central pair of permanent incisors is seen and the depression in the temporary corners is disappearing. The medical pair of permanent incisors comes up at three and a half years. According to Capt. Hayard, I.A.V.C., through whose courtesy information on buffaloes has been obtained, the date of eruption of laterals is very variable because some animals develop six permanent incisors at three and a half years, while others have only four permanent molars at four years. At six years, all the permanent incisors are up, while the corner incisors develop a hook at the seventh year and the centrals show a star (enamel line). At eight years, the corner incisors appear more worn; the hook of the wearing surface disappears and the wearing surface of the central, middle and lateral incisors begin to show the star: from the front view, the teeth appear longer and more ridged. The molar teeth were not examined.

The horns give no indication of the age in adult buffaloes as individual rings cannot be definitely distinguished.

It is ovserved that the teeth in our cattle erupt at a later stage than those of English ones. In common English cattle brought up under ranch conditions, according to Miller and Robertson [1937] the first pair of incisors is up at one year and nine months to two years, the second at two and a half years, the third at three years and the fourth at three and a half years, to four years. In the case of molars the fourth permanent molar arises at six months, the fifth at one year and three months to one and a half years, the sixth at two years, the first and second at two and a half years, and the mouth is complete at three and a half years, to four years. Robertson and Miller's figures for ranch cattle and Ellenberger and Baum's figures indicating the ages of eruption of permanent teeth for breeds maturing late, correspond to some extent with those obtained in the case of our cattle, and are given in the Table II.

TABLE II

Permanent dentition

Teeth	Indian cattle	Foreign cattle (*vide* Miller an Robertson)			Breeds maturing late (*vide* Ellenberger and Baum)		
		Highly bred stock	Commonly bred stock	Ranch cattle	Early	Medium	Late
	Month	*Months*			*Months*		
First Incisor	24—30	21	21—24	24	17	21	25
Second ,, ...	36	27	30	30—36	22	27	32
Third ,, ...	48	33	36	42	32	36	40
Fourth ,, ...	54—60	39	42—48	54—60	36	45	52
First molar	24	24	30	24	24	26	28
Second ,, ...	24	24	30	24	24	26	28
Third ,, ...	36	33	36	33	28	31	34
Fourth ,, ...	6	6	6	6	5	5	
Fifth ,, ...	18	12—15	15—18	12—15	15	16	18
Sixth ,, ...	24	21	24	21	24	26	8

ACKNOWLEDGMENTS

Thanks are due to the various provincial Directors and Livestock Officers for sending data from the provincial farms, and to Lt. Col. J. Clabby for supplying information on buffaloes collected by Capt. Hayward, and to Mr Mathur for collecting data from the Indian Agriculture Research Institute herd, New Delhi.

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THE DIGESTIBLE NUTRIENTS OF CERTAIN CEREAL GRAINS AS DETERMINED BY EXPERIMENTS ON INDIAN FOWLS

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(Received for publication on 26 August 1947)

In the absence of information on the digestibility of certain Indian poultry feeds the authors have determined the digestible nutrients of three comparatively cheap cereal grains *cheena*, *jowar* (*Sorghum vulgare*) and *ragi* (*Eleusine coracana*). Experiments on two other grains, viz. barley and *bajra* (Pennesetum typhoideum, bulrush millet), for which foreign data are available, have also been conducted to see if the figures given by western workers hold good for similar Indian grains. Except *ragi*, the grains studied are sparingly used for human consumption in India. An investigation of their value as poultry-feeds is necessary since such staple cereals as rice and wheat are often in short supply in this country and should be used sparingly in rations for livestock.

Katayama [1924] and Halnan [1926] for obvious reasons, used indirect methods of determining digestibility coefficients of poultry feeds by analysing the mixed excreta and making use of certain equations. These equations are given by Katayama (*Loc. cit*) and are based on data obtained on only two birds, before and after operation. The formulae as reproduced in an article by Halnan [1926] are as follow:

1. Dung Nitrogen=Excretory N-(Uric acid N-free ammonia N) 114·6 per cent/102·3 per cent
2. Urinary Nitrogen=Excretory N-Dung N
3. Urinary organic matter=Urinary N × 3·26
4. Urinary ether extract=Organic urinary matter × 108 per cent

This method of computation has been used in this study. Fraps [1920] has determined the digestibility of a large number of feeds taking the urinary products as consisting entirely of uric acid and ammonia. Though this method has been used by a number of other workers it is undoubtedly less accurate.

Experimental

The cereal grains were fed singly, without the addition of any other feed to adult *desi* (indigenous) cockerels between 1 and 1½ years of age. They were housed individually in roomy (16 × 12 × 16 in.) metabolism cages with wire bottom. The mixed excreta were collected on polished glass-plates placed under each cage. Food was provided in wooden boxes and water in triangular tins fastened to the sides of the cages. The birds were kept in their respective cages for a few weeks before the experiment so that they become used to the environment. Each metabolism experiment was preceded by a preliminary period of five days with a collection period of seven to eight days. The birds were weighed on two days, viz. at the commencement and end of the digestibility trial, the average weight being used for calculation. During the metabolism experiment a weighed quantity of the test feed, the amount of which was based on the consumption of each bird during the preliminary period was offered to the cockerels. Any food left over was weighed. Every attempt was made to feed an adequate amount of diet and to ensure an uniform intake throughout the period of observation. The birds were given 1 gm. of common salt and 2 gm. of limestone-grit along with their daily diet and water was always available to them. Every morning, the excreta were scraped and collected in glass-stoppered bottles after mixing with 10 c.c. of 10 per cent acetic acid after collection. The glass stoppered bottles were kept at 30°F to arrest fermentation. At the end of the experimental period the pooled sample was rendered distinctly acidic by adding acetic acid, mixed thoroughly and weighed. Suitable quantities were then weighed out into tared porcelain dishes and dried in an electric oven at a temperature not exceeding 70°C. The dried material was ground finely, passed through a sieve to remove any pieces of feather and stored in glass-stoppered bottles for subsequent analysis.

The total nitrogen was estimated by the Kjeldahl method while the ether extract, fibre and ash were determined by the A.O.A.C. procedure. Bose's method [1944] was used for uric acid and ammoniacal nitrogen was obtained by following Van Slykes aeration method using a mixture of methylene blue and methylred [deWasselow] as indicator [Cole 1933].

Results and discussion

The composition of the grains used is given in Table I and the digestibility figures in Table II. Of the five cereals studied only two viz., barley and *bajra* (bulrush millet) have been studied by previous workers Fraps [1928] and Halnan [1928] and the results are compared in Table III. Table IV contains an abstract of the results and the values for digestible protein and starch equivalent. The starch equivalent was calculated in the usual manner using the normal figures.

Individual variation.

With *cheena* there is little variation in the digestible coefficients among individual birds, except for crude fibre which is not of much importance in poultry. In the case of *ragi* the digestibility figures for protein vary fairly widely while those for crude fibre are erratic as in other cases. Barley showed a comparatively wide variation in the digestibility coefficients of crude fat and nitrogen free extract. This may be due to the fact that barley is not very palatable to poultry and the food intake and faecal output are both irregular. The individual figures for *bajra* and *jowar* tally except in respect of crude fibre. The crude fibre of *jowar* does not seem to be digested at all.

Table I

Percentage composition of experimental feeds

Feed	Moisture per cent	Crude protein per cent	Crude fat per cent	N.F.E. per cent	Crude fibre per cent	Ash per cent
Cheena	11·68	10·09	1·20	65·28	8·17	3·58
Ragi	12·26	9·83	1·47	70·21	3·32	2·91
Barley	12·12	11·24	1·19	66·20	5·52	3·73
Bajra	12·50	8·33	4·10	72·02	1·08	1·97
Jowar	13·53	6·19	2·01	74·82	1·65	1·80

Table II

Digestibility coefficients of the feeds

Feed	Bird No.	Organic matter per cent	Crude protein per cent	Crude fat per cent	Crude fibre per cent	N.F.E. per cent
Cheena	1	81·60	84·28	76·98	11·31	90 29
	2	80·89	85·61	71·20	5·18	87·96
	3	79·89	84·37	76·63	10·47	88·33
	4	80·64	86·52	81·32	9·67	89·17
	5	80·72	87·04	80·46	5·49	89·58
	6	79·68	78·93	78·34	0·04	89·33
	7	79·22	77·47	74·08	4·35	89·21
	Average	80·38	83·32	77·00	7·50	89·12

TABLE II—*contd.*

Feed	Bird No.	Organic matter per cent	Crude protein per cent	Crude fat per cent	Crude fib e per cent	N.F.E. per cent
Ragi	1	86·58	90·08	68·03	29·61	86·82
	2	86·72	88·57	64·18	36·38	83·64
	3	82·43	70·20	70·31	12·73	84·83
	4	83·41	82·60	67·20	17·78	85·85
	5	83·09	68·69	71·24	13·82	86·02
	6	81·95	66·55	68·03	10·16	84·30
	Average	84·03	77·77	68·16	20·08	85·24
Barely	1	76·52	72·38	16·35	13·86	78·63
	2	79·06	71·27	11·59	22·20	63·80
	3	76·86	80·79	21·41	41·99	80·71
	4	75·97	72·62	33·59	13·31	77·58
	5	85·19	82·06	43·60	44·53	90·33
	Average	78·72	75·82	25·31	27·18	78·21
Bajra	1	85·02	93·40	53·10	..	87·28
	2	87·48	83·90	61·34	7·82	90·65
	3	87·02	81·68	60·43	6·72	90·51
	4	89·06	87·10	69·90	5·51	91·77
	5	88·51	89·45	77·04	..	90·51
	6	86·04	87·04	62·30	..	88·76
	Average	71·98	87·10	64·17	3·34	90·00
Jowar	1	88·28	99·74	71·19	..	89·93
	2	88·12	99·68	70·30	..	90·16
	3	87·12	72·38	64·21	..	91·26
	4	88·31	89·28	74·98	..	91·31
	5	85·98	93·74	61·44	..	90·99
	6	88·78	93·48	72·02	..	91·11
	7	88·00	88·90	66·68	..	90·72
	Average	87·80	91·03	68·69	..	90·78

TABLE III

Comparison of present observations with those available in literature

		Organic matter per cent	Crude protein per cent	Crude fat per cent	Crude fibre per cent	N.F.E. per cent	D.P. per cent	S.E. per cent
Barley	Fraps	..	72·00	58·10	10·80	82·10	8·64	65·48
	Present authors	78·72	75·82	25·31	27·18	78·21	8·52	63·43
Bajra	Halnan	86·0	90·4	72·7	5·00	88·00	11·7	78·4
	Present authors	87·2	87·1	64·2	3·3	90·00	7·3	76·5

TABLE IV

Digestible constituents

	Organic matter per cent	Crude protein per cent	Crude fat per cent	Crude fibre per cent	N.F.E per cent	D.P. per cent	S.E. per cent
Cheena	80·38	83·32	77·00	7·50	89·12	8·00	65·31
Ragi	84·03	77·77	68·16	20·08	85·24	7·27	66·40
Barley	78·72	75·82	25·31	27·18	78·21	8·52	63·43
Bajra	87·19	87·10	64·17	3·34	90·00	7·25	76·48
Jowar	87·80	91·03	68·69	..	90·78	5·36	75·46

Indian and foreign figures

There is close agreement between the values for barley obtained by us and those reported by Fraps (*Loc. cit*) except for crude fat and crude fibre. Since barley is poor in both these constituents the difference in their digestibility figures had little influence on the values of starch equivalent which agreed well. In the digestibility of *bajra*, though there is a slight difference between our figures and those of Halnan [1926] in respect of protein the rest of the digestibility coefficients are closely comparable. For digestible protein our figure is lower than Halnan's which may be due to the difference in protein content of the samples, while the starch equivalent values agree. The similarity between foreign and Indian figures of starch equivalent and digestibility coefficient of protein suggest that the former may safely be used in formulating rations under Indian conditions.

Comparative values of the grains

In comparing the energy values of grains used in the present investigation the digestible coefficient of nitrogen free extract should be the main consideration since the grains provide minute quantities of digestible fat and fibre and the digestible protein is responsible for only a small fraction of the available energy. On this basis the grains may be arranged in the following order according to their net energy value ; *bajra* and *jowar* are superior ; *cheena* and *ragi* are of medium quality while barley is the poorest. Further, *bajra* and *jowar* are equally good and the value of *cheena* does not differ significantly from that of *ragi*. It is remarkable that the published figures for starch equivalent of wheat [Halnan, 1928] compare favourably with that of *bajra* and *jowar* ; hence these millets may very well replace wheat in poultry rations. Of course such rations should be adjusted in other respects and tested carefully before adoption on a large scale.

The protein values of *ragi*, *jowar* and *bajra* are comparatively low (Table 1). Although individual samples of the same grain may differ fairly widely in protein content. The available Indian data show that the above millets have a lower protein content than wheat, barley, maize or oats. Consequently in spite of the high digestibility coefficients of protein, the three millets rank comparatively low in digestible protein. The digestible protein of *jowar*, however, does not represent the appropriate value for this millet since our sample contained less protein than is generally found in Indian samples. On this basis it can be classed with *bajra* and *ragi* in respect of digestible protein and may be considered inferior to barley or *cheena*. Barley is richer than the other four grains in digestible protein, while *cheena* is of intermediate value. It is recognized that the digestible protein figure alone is insufficient to enable one to judge the value of a feed or ration as a source of available protein.

SUMMARY

The digestible nutrients as poultry feed of five common cereal grains, *cheena*, *ragi*, barley, *bajra* and *jowar* have been determined. The starch equivalent and digestible protein were calculated from these data. *Bajra* and *jowar* were found to be equally superior to the other three grains. Regarding their starch value they are indeed as rich as wheat. Barley was found to be the poorest in net energy content while *ragi* and *cheena* were of intermediate quality.

The digestibility coefficients and starch equivalent figures for *bajra* and barley agreed well with those obtained by Western workers.

The grains arranged in decreasing order of digestible protein content stand as under : barley, *cheena*, *bajra* or ragi or jowar, the last three millets being of equal value, and barley the richest of all.

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THE MANGANESE CONTENT OF SOME COMMON POULTRY FEEDS

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(Received for publication on 26 August 1947)

IN view of the importance of manganese in poultry nutrition, the manganese content of some poultry feeds, commonly used in India, was determined to see if rations compounded from them are sufficient with respect to their manganese content. Though figures for some of these feeds used in foreign countries are available, the Indian products have been examined for comparison in order to find out if they show any variation. The manganese content of some Indian feeds have been determined, in a different connection, by Rudra [1939] and Sundara Rao [1940]. We have included in our list some feeds studied by these workers to see if any differences exist ascribable to soil variations.

MATERIALS AND TECHNIQUE

The cereals and their by-products were purchased locally except *ragi* which was obtained from Madras. As far as possible only the portions of leafy vegetables which are not used for human consumption have been used for the estimation. These and other grasses were obtained from the institute farm.

The A.O.A.C. (1940) method using ammonium persulphate was employed in several cases. In cases, however, where the concentration of chlorides was high, the method of Willard and Greathouse [1917] with slight modifications was employed, as in our experience the latter method produced a colour which was brighter and stable for longer periods, while the colour produced by the persulphate method was somewhat dull and faded out quickly. The modified method of Willard and Greathouse was as follows:

The sample was ashed in a silca crucible at dull-red heat. The ash was dissolved in a small quantity of concentrated nitric acid, about 5 c.c. of syrupy phosphoric acid and sufficient quantity of distilled water were added till the crucible was about three-fourths full. The solution was heated for about 30 minutes on a water bath for complete extraction of the ash, then cooled, filtered and washed. To the filtrate, in a 150 c.c beaker, about 0·3 g. of potassium periodate was added and the beaker heated on water bath till the maximum colour developed. The solution was cooled and compared in a colorimeter with a standard solution of manganous sulphate similarly treated. In preliminary experiments to compare the above procedure with the A.O.A.C. [1940] method using ammonium persulphate, agreement was found to be fairly close.

RESULTS AND DISCUSSION

The results of the determination are given in Table I which shows that certain cereal by-products, such as rice bran and wheat bran are excellent sources of manganese. *Ragi* used in South India by human beings and for poultry is also a good source of this element. The low manganese in polished rice may be due to milling. *Bajra*, *cheena*, *jowar* and barley are poor sources and when the ration consists entirely of these, the inclusion of necessary supplements in the form of manganese sulphate or other concentrates may be necessary to get the optimum level of manganese. The cakes of linseed, groundnut and mustard seem to be moderate sources. Limestone is a good source but there is much variation from sample to sample. Among the green feeds studied cowpeas appear to be a very good

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TABLE I

Manganese contents (p. p. m.) of food stuffs

Cereals and their by products.		
Bajra	(*Pennisetum typhoideum*)	12·9
Barley	(*Hordeum vulgare*)	18·4
Jowar	(*Sorghum vulgare*)	12·2
Maize (yellow)	(*Zea Mays*)	10·3
Cheena	....	13·7
Oats	(*Avena sativa*)	37·5
Paddy	....	61·6
Ragi	(*Eleusine coracana*)	99·2
Rice (milled)	(*Oryza sativa*)	11·5
Rice bran	(*Oryza sativa*)	156·0
Wheat	(*Triticum vulgare*)	33·0
Wheat bran	(*Triticum vulgare*)	101·1
Pulses		
Bengal gram (with outer husk)	(*Cicer arictinum*)	29·7
Bengal gram (without outer husk)	....	21·4
Cow peas	(*Vigna catiang*)	20·3
Lentil	(*Lens esculenta*)	13·4
Peas	(*Pisum sativum*)	15·2
Soyabean (black)	(*Glycine hispida*)	32·1
Oil-seed cakes		
Ground nut cake	(*Arachis hypogea*)	45·1
Linseed cake	(*Linum usitatissimum*)	57·0
Mustard cake	(*Brassica juncea*)	43·0
Leafy vegetables, grasses, etc.		
Amaranth	(*Amaranthus gangeticus*)	47·7 (dry)
Cabbage	(*Brassica oleracea capitata*)	6·0 „
Carrot leaves	(*Daucus carota*)	12·0 „
Cauli flower leaves	(*Brassica oleracea botrytes*)	4·7 „
Knol-Kohl leaves	(*Brassica oleracea caulorapa*)	13·0 „
Cow pea leaves	(*Vigna catiang*)	84·6
Guar leaves	(*Cyanopis psoraloides*)	61·3 „
Jowar leaves	(*Sorghum vulgare*)	37·0 „
Elephant grass	(*Pennisetum purpureum*)	13·3 „
Guinea grass	....	23·5 „

TABLE I—*contd.*

Miscellaneous		
Limestone	....	84·0
Meat offals	....	3·6 (fresh) 17·3 (dry)
Skimmed milk	....	0·8 (dry)
Meat scrap	....	Trace.

TABLE II

Manganese contents compared with those found out by other workers

Feed	Skinner and Peterson (1928)	Schaible, Bandemer and Daridson (1938)	Rudra (1939)	Sundara Rao (1940)	Present authors
Barley	19·0	14·0	..	..	18·4
Oats	31·8	36·0	..	..	37·5
Rice (milled)	12·0	13·0	dry 13·4	12·6	11·5
Wheat	54·5	31·0	dry 20·7	36·4	33·0
Wheat bran	140·4	108·0	..	..	101·1
Bengal gram	..	..	..	28·6	29·7
Soyabean	29·5	32·0	..	..	32·1
Peas	..	..	..	18·1	15·2
Amaranth	..	..	..	60·2	47·7

Source. Another interesting point is that in general the leaves are richer in this element than the corresponding grains of *jowar* and cowpeas. In Table II our results are compared with those of other workers. Agreement in several cases seems to be good. It has been generally advocated that 40-50 p.p.m. of manganese is the optimum level in poultry rations. With Indian samples we have found that the manganese content of a ration, which does not contain sufficient rice bran or wheat bran, falls below 50 p.p.m. Since no case of manganese deficiency has been reported from any scientifically managed farms in India, it may be supposed that such difficiency is unlikely to occur when the ration is otherwise balanced.

SUMMARY

The manganese content of 35 substances used as common poultry feeds in India has been determined by a colorimetric method.

Of cereal by-products, wheat-bran and rice-bran are excellent sources. *Bajra, jowar, cheena* barley and polished rice are poor sources.

The manganese contents of some Indian poultry-feeds have been compared with those already reported and the agreement is found to be good.

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STUDIES OF THE BIOLOGICAL VALUES OF THE PROTEINS OF CERTAIN POULTRY FEEDS

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(Received for publication on 26 August 1947)

There is a little information on the nutritive vaue of the proteins of certain cereal grains fed either singly or in combination to poultry in India. The biological value of some of these grains for rats is known ; but the value of a protein for poultry must be assessed by conducting experiments on the birds themselves since their amino acid requirements are different from those of rats [Almquist, 1941]. The lack of information on the nutritive value of proteins for poultry is due mainly to the fact that urine and faeces are excreted through a common opening and their separate collection is impossible unless artificial means are adopted. The following review indicates the methods used to tide over this difficulty.

Fields and Ford [1900] and Brown [1904] assumed that the sum of uric acid and ammonia nitrogen represented the urinary nitrogen and they subtracted this sum from the total nitrogen to get the faecal nitrogen. This method is not sound since uric acid and ammonia nitrogen form only a part of the urinary nitrogen. Coulson and Hughes [1930], and St John, Johnson, Carver and Moore [1932] calculated the urinary nitrogen by taking the sum of uric acid and ammonia nitrogen as 80 per cent of the total urinary nitrogen. The flaw in this method is , the arbitrary assumption of the factor 80 per cent. Paraschtshuk [1902]. Lehmann [1904] and others produced an aritficial anus by operation and collected the faeces and urine separately. This method has been criticized because the bird is under a strain. Katayama [1924] used a correction factor based on analysis of mixed excreta of normal birds, and of urine and faeces of the same birds after operation. but this cannot be regarded as accurate since the experiment was conducted on only two birds. The method of Heller Morris and Shirley [1930] based on the mechanical separation and analysis of the 'white caps' cannot be expected to yield accurate results. The urine residue found in the front of a normal excretion is commonly known as ' white cap '. Mitchell [1924] proposed a method, later elaborated by Ackerson, Blish and Mussehl [1926] to determine the efficiency of dietary protein. The nitrogen excretion of birds on a diet practically free from nitrogen was first determined for calculating the biological value of the protein. Ackerson, Blish and Mussehl [1929] thus obtained the comparative efficiency of various proteins of cereals when fed to poultry at varying levels of nitrogen in-take. Macdonald and Bose [1944] also employed this method in principle and we have used it with slight modifications in these investigations where the efficiency of proteins of five cereals, viz. *cheena*, *jowar*, *bajra*, *ragi* and barley, commonly used as poultry feeds in India has been determined.

Experimental

The nitrogen balance trials were carried out on adult *desi* (indigenous) cockerels. The feeding, management and other experimental technique was the same as in our previous study [Mukherjee and Parthasarathy (in press)]. The experimental rations consisted of a single kind of grain with daily supplements of about 1 g. of common salt and 2 g. of limestone grit per bird. The metabolism periods were of 10 days and were preceded by adjustment periods of five to seven days. Throughout each metabolism experiment, the birds were fed a constant amount of food sufficient to meet the energy requirement and maintain body weight. The endogenous and metabolic nitrogen of the birds was determined by feeding them on a nitrogen-free diet composed of sago, common salt, limestone-grit, potassium phosphate and supplements of ' Adexolin ' and ' Berin '. (These are patents of Glaxo Laboratories which were fed orally, 1 drop of ' Adexolin ' daily and 1 c.c. of a suspension of 1 tablet of ' Berin ' in 5 c.c. of distilled water, per bird.) The metabolism and adjustment periods of the nitrogen-free experiment were seven and five days respectively. This diet was well relished by the birds, and they maintained weight during the experiment Seven birds were fed the

nitrogen-free diet and the total nitrogen in the excreta, uric acid nitrogen and ammonia N were determined. The endogenous N and faecal metabolic nitrogen were calculated by Coulson and Hughes' formula (*Loc. cit*).

Total N of dried excreta was determined by the *Kjeldahl* method. The ammonia N was estimated by the *Van Slyke* aeration method using a mixture of methylene blue and methyl red as indicator Cole [1933]. To 1 *g.* of the excreta 3-5 of potassium carbonate and a few c.c. of distilled water were added and the aeration carried out for three hours. Parallel experiments with and without acidification of the excreta with HCl before adding potassim carbonate, showed that subsequent acid treatment was not necessary when the excreta was dried after acidification with acetic acid. The uric acid was estimated as by Bose [1944].

The urinary nitrogen was calculated on the basis that uric acid and ammonia N represented 80 per cent of total urinary nitrogen. The faecal nitrogen was obtained by subtracting the computed value of urinary nitrogen from the total nitrogen. In the case of *jowar*, it was assumed, for the reasons mentioned later on that the sum of uric acid and ammonia nitrogen formed 90 per cent of the urinary nitrogen.

The results were calculated according to the following formulae Mitchell [1924].

(1) $\frac{\textit{Food N-(Faecal N-Metabolic N)}}{\text{Food N}} \times 100 =$ True coefficient of digestibility.

(2) $\frac{\textit{Food N-(Faecal N-Metabolic N)} - \text{(urinary N-endogenous N)}}{\text{Food N-(Faecal N-Metabolic N)}} \times 100 =$ Biological value.

Discussion

The metabolism data on nitrogen-free diet (Table I) show that the average endogenous N was 85 mg. per kg. body weight and the average metabolic faecal nitrogen was 0·0884 g. per 100 g. dry matter in-take. In these studies each nitrogen feeding period was not preceded by a nitrogen-free period. This step was omitted since according to Ackerson *et al* [1929]. Individual standardization of the subject produces no greater accuracy of results than the use of an average value for the endogenous nitrogen excretion in calculating biological values. It has been assumed that in birds as in animals the metabolic faecal nitrogen is proportional to the dry matter in-take and that endogenous N is proportional to body weight. The average values thus calculated have accordingly been used in the computation of endogenous and metabolic nitrogen excreted during the nitrogen feeding periods for the calculation of biological values.

The compostion of the cereal grains in these experiments is given in Table II. The biological values (Tables III-VI) are calculated on the assumption that the uric acid nitrogen and ammonia nitrogen represent 80 per cent of the total urinary nitrogen. But, in the case of *jowar* (Table VII) this assumption gave negative values for faecal nitrogen which strengthens the doubt of Van Landingham, Clark and Schneider [1942] whether with all diets uric acid and ammonia N represent a constant percentage of the total urinary N. We have therefore assumed, purely on an arbitrary basis, that the sum of uric acid N and ammonia N forms 90 per cent of the total urinary nitrogen on the *jowar* diet and thus obviated the difficulty of negative values for faecal nitrogen. Macdonald and Bose determined the biological values of three mashes for which the 80 per cent basis might possibly hold good as in the case of the remaining four feeds in the present observation. More extensive studies with a larger number of feeds are required to settle this point. The results obtained for *jowar* show that the uric acid N and ammonia N do not remain a constant percentage of the urinary nitrogen with all diets.

TABLE I

Metabolism data on non-nitrogenous diet

Bird No.	Body weight kg.	Food dry matter in-take per kg. body weigh	Total nitrogen excreted per kg. body weight	Urinary nitrogen 1·25 × (Uric acid + ammonia N) per kg.	Faecal nitrogen per cent
1 . .	1·80	21·8	0·080	0·065	0·015
2 . .	2·57	27·9	0·141	0·114	0·027
3 . .	2·37	27·3	0·107	0·083	0·024
4 . .	2·57	24·6	0·126	0·099	0·027
5 . .	1·86	24·6	0·095	0·067	0·028
6 . .	2·77	20·9	0·079	0·068	0·011
7 . .	2·11	24·0	0·115	0·096	0·019
Average .	2·29	24·4	0·106	0·085	0·022

TABLE II

Percentage composition of experimental feeds

Feed	Moisture per cent	Ash per cent	Crude protein per cent	E. Extract per cent	Crude fib per cent	N. F. E. per cent
Ragi	12·3	2·9	9·8	1·5	3·3	70·2
Cheena	11·7	3·6	10·1	1·2	8·2	65·3
Bajra	12·5	2·0	8·3	4·1	1·1	72·0
Barley	12·3	3·9	11·9	1·0	5·1	65·7
Jowar	13·5	1·8	6·2	2·0	1·6	74·9

TABLE III

Nitrogen metabolism experiment with ragi (metabolism figures are given on daily basis)

Bird No.	Food N gm.	Faecal N gm.	Urinary N gm.	N balance	Biological value per cent	True dig. coeffi. per cent
1 . .	1·416	0·3783	0·4310	0·6067	82·24	78·23
2 . .	1·305	0·3551	0·2795	0·6704	92·62	77·71
3 . .	1·307	0·3791	0·3826	0·5453	81·43	75·37
4 . .	1·305	0·3997	0·3193	0·5860	86·48	74·27
5 . .	1·232	0·2241	0·5950	0·4129	73·31	86·76
6 . .	0·7997	0·2578	0·3468	0·1951	69·85	73·36

TABLE IV

Nitrogen metabolism experiment with cheena (metabolism figures are given on daily basis)

Bird No.	Food N gm.	Faecal N gm.	Urinary N gm.	N balance	Biological value per cent	True dig. coeffi. per cent
1 . .	1·694	0·202	0·951	0·541	53·23	92·90
2 . .	1·678	0·170	0·987	0·521	50·73	94·64
3 . .	1·016	0·123	0·513	0·380	64·59	92·83
4 . .	1·226	0·144	0·694	0·388	55·40	92·90
5 . .	1·688	0·156	0·967	0·565	54·66	95·72
6 . .	1·307	0·220	0·816	0·271	52·31	87·92
7 . .	1·452	0·282	0·730	0·440	58·62	85·39

TABLE V

Nitrogen metabolism experiment with bajra (metabolsim figures are given on daily basis)

Bird No.	Food N gm.	Faecal N gm.	Urinary N gm.	N balance	Biological value per cent	True dig. coeffi. per cent
1 . .	1·070	0·1014	0·3040	0·6646	93·71	96·34
2 . .	1·106	0·1283	0·2927	0·7450	92·45	77·16
3 . .	1·198	0·2154	0·4735	0·5091	77·02	87·88
4 . .	1·137	0·2145	0·2682	0·6543	93·46	87·26
5 . .	0·977	0·2252	0·4153	0·3375	73·31	82·79
6 . .	0·910	0·1534	0·3252	0·4314	82·83	89·02

TABLE VI

Nitrogen metabolism experiment with barley (metabolism figures given on daily bassis)

Bird No.	Food N gm.	Faecal N gm.	Urinary N gm.	N balance	Biological value	True dig. coeffi. per cent.
1 . .	2·082	0·5241	0·4493	1·1076	85·37	78·73
2 . .	1·796	0·4783	0·3215	0·9962	90·65	77·21
3 . .	1·338	0·2392	0·6342	0·4646	61·91	86·16
4 . .	1·070	0·2680	0·2619	0·5401	89·80	78·98

TABLE VII

Nitrogen metabolism experiment with jowar (metabolism figures are given on daily basis)

Bird No.	Food N gm.	Faecal N gm.	Urinary N gm.	N balance	Biological value	True dig. Coeffi per cent
1 . .	0·4556	0·1121	0·2080	0·0375	63·42	83·34
2 . .	0·6116	0·0557	0·3996	0·1563	69·82	98·51
3 . .	0·6207	0·0842	0·5259	0·0006	47·18	74·21
4 . .	0·6509	0·0549	0·4647	0·1313	57·28	99·22
5 . .	0·8798	0·0746	0·5740	0·2312	43·94	96·78

The average biological values together with the standard errors and the true digestibility coefficients are given in Table VIII. Alongside are also given the biological values as determined by rat experiments [Aykroyd, 1940]. It appears that *bajra*, *ragi* and barley have nearly the same biological value. The biological value of *bajra* was the same for rats and fowls but in the case of the other three cereals the values for the two species differed markedly. These observations support the view of Almquist (*Loc. cit*) that the amino acid requirements of the fowl are different from those of the rat and that the values obtained with the latter do not hold good for the former.

TABLE VIII

Summary of results obtained

Feed	Biological value per cent		True dig. coeffi average per cent	Biological value for the rat Aykroyd 1940	Protein value per cent
	Mean	S. E.			
Ragi	80·99	3·15	77·61	89	63·2
Cheena	55·65	1·60	90·33	..	50·4
Bajra	85·46	3·51	86·74	82	74·0
Barley	81·93	5·85	80·27	71	65·6
Jowar	56·33	4·33	94·41	83	52·6

The digestibility coefficients of *jowar*, *cheena* and *bajra* are fairly high and those of barley and *ragi* are low. In protein values (which take into consideration both the biological values and the digestibility coefficients *bajra* is the richest, barley and *ragi* coming next. while *jowar* and *cheena* are the poorest. According to Halnan also, *bajra* (bulrush millet) is a very good food and is akin to wheat in feeding value. Further work with laying birds and growing chickens is necessary to obtain more evidence on this point.

SUMMARY

The biological values and digestibility coefficients of the proteins of five cereal grains, *jowar*, *cheena*, *bajra*, *ragi* and barely used commonly as poultry feeds in India have been determined by the balance method using adult cockerels for experiment. The average biological values are 56, 56, 85, 81 and 82 per cent respectively. In protein value *bajra* is found to be the best, barley and *ragi* next best and *jowar* and *cheena* the poorest. Evidence is presented to show that the sum of uric acid nitrogen and ammonia nitrogen does not remain a constant percentage of the urinary nitrogen with all diets.

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ABSTRACTS

WILLIAM ORR (1945). **Observations on Rinderpest in Goats Imported to Malaya** *Jour. Comp. Path. & Ther.* **55**, 185-200

SOME years before 1939, the Malayan governments prohibited the importation of cattle, sheep and goats from India on account of the danger of introducing rinderpest and other diseases. This measure, together with the constant vigilance of the veterinary departments, enabled these governments to eradicate rinderpest from Malaya and only occasional localized outbreaks of the disease due to the introduction of infection from other countries continued. In 1939, however, military requirements made it necessary to relax these restrictions and to import large numbers of goats from India. When consignments were expected to arrive Singapore was overcrowded with highly susceptible Bali-oxen, which had been imported to supplement the local stock of food. The indigenous water-buffalo of Malaya is also highly susceptible to rinderpest. The consequences of introducing the disease to this susceptible stock would have been disastrous specially at a time when conservation of existing food was of major importance.

The consignments of goats from India arrived at Singapore and rinderpest was detected in them on arrival. So, without regard to the immediate losses, it was decided to eradicate this infection the slaughter and disposal of all the animals in the shipments involved.

Four consignments arrived, comprising 1,500 goats in all. The disease manifested itself in an unusually virulent form and caused heavy mortality. In one of these four consignments, rinderpest was not diagnosed immediately on arrival at the port. The disease however, made its appearance within 24 hours of the landing of the animals and rapidly assumed epizootic form within the next 48 hours.

Of the first consignment of 500 goats shipped at Calcutta, 40 died on the voyage, 191 died within eight days of arrival and the rest were destroyed. In the second consignment of 500 goats 72 died on the way, 272 died in seven days after arrival and the rest were destroyed in a moribund state. In the third consignment of 200 goats shipped from Calcutta two died of other causes on the voyage and the remaining 198 goats showed no evidence of disease on arrival. But the disease was noticed the next day. Six animals died within two days after arrival and on the third day the remaining animals including 19 sick animals were all slaughtered. In the last consignment of 300 goats shipped from Calcutta 16 animals were lost on the voyage, 19 died within two days after arrival and the rest including 37 sick animals were all destroyed.

Rinderpest was detected in a consignment of goats shipped from Calcutta to Penang at about the same time. Biological tests carried out with material obtained from this batch of goats in Bali-oxen and indigenous goats in Penang showed that this rinderpest virus was highly infectious and virulent. The peracute and fatal reactions that followed in the experimental animals were essentially similar to those observed in these animals following infection with rinderpest virus of bovine origin. The symptoms and post-mortem lesions observed in these goats at Singapore are described in great detail. The author also refers to Whitworth's account of an undiagnosed disease which occurred among Indian goats imported to Singapore in 1935 and which was suspected as rinderpest and compares it with the disease that occurred in 1941.

After this outbreak, it was decided to discontinue further importation of Indian goats to Malaya pending a revision of the conditions under which the traffic might be resumed. But the outbreak of hostilities in Malaya in December, 1941, prevented the resumption of importation. [P.R.K.J.]

The Value of Vitamin a Therapy in Cases Diagnosed as Ketosis in Dairy Cows

C. E. HAYDEN, M. G. FINCHER, S. J. ROBERTS, W. J. GIBBONS AND A. G. DANKS (1946). *The Cornell Veterinarian* **36**, *71*

EVER since Patton made the first observation in 1944 that vitamin A deficiency is responsible for ketosis, considerable attention has been paid to this treatment.

The authors report their findings on nine dairy cows. Six of them were uncomplicated cases of ketosis, one of mild ketosis complicated with metritis and cystic ovary and two of mild ketosis complicated with enteritis. Six to 18·5 million units of vitamin A were given to the cows without any ameliorative effect. The authors conclude that vitamin A therapy in ketosis is of no value. A big dose had no favourable effect on the blood sugar and acetone bodies of the blood and urine. [N.D.K.]

The Importance of Commercial Processing for the Protein Value Food Products. H. H. Mitcheli, T. S. Hamilton and J. R. Beadles (1945).

J. Nut. 29,1,13—25

THE authors have determined the effect of heat treatment on the protein of the soya bean and the nutritive value of meal obtained after the commercial extraction of oil from oil bearing seeds like coconut and sunflower. The autoclaving of soya bean for one hour under 15 lb. pressure increased, digestibility of its nitrogen by 5 per cent and the biological value by 18 per cent. The effect is attributable to improved availability of the amino-acid, cystine, contained in the soya bean protein.

Further study of the effect of heat treatment on the nutritive value of the protein of the soya bean has been made with growing rats using the nitrogen balance method on three samples of soya bean flour (1) raw flour, not submitted to any treatment other than grinding (2) exploded flour, where the beans were placed in a rotating closed chamber at a temperature of 550°F for 90 seconds, steam being then injected into the chamber for an additional period of 60 seconds until the pressure reached 185 lbs. per square inch followed by the suddenly opening of the chamber to the atmosphere (3) partially exploded flour, prepared exactly as (2) except that the steam was injected over a period of 45 seconds till the pressure was 165 lbs. per square inch. Although the results of chemical analysis of the three samples were remarkably similar the average digestibility and biological value of the nitrogen of the raw soya bean flour, 84·8 and 59·4 respectively, were significantly lower than those of the fully exploded flour, 93·4 and 71·2 respectively which in turn were lower than that of the partially exploded flour which were 95·6 and 75·2.

In the commercial preparation of coconut meal and sunflower seed meal, the authors have adopted a solvent extraction method at a temperature not exceeding 75°C and found that it produces uniform meals, stable in character, and with the original nutrients unimpaired in amount and quality. Tests carried out on the coconut meal show its protein to be 86 per cent digestible and to possess a biological value of 71, which are considerably higher than that obtained for a product tested earlier but prepared by the usual drastic methods. The protein of sunflower meal prepared in the same way, was 84·5 per cent digestible with a biological value of 64·5. Taking the protein content, the digestibility of protein and the biological value of the digested protein into consideration, which according to the senior author constitute the net protein value, the products tested fall into the following order.

Sunflower seed meal (33·7), partially exploded soya flour (30·2), fully expolded soya flour (27·8) raw soya flour (21·4) and coconut meal (12·6). The method of oil extraction at low temperatures has, therefore, many advantages in preserving the high nutritive value of the meals of many oil bearing seeds. (N.K.)

DOUGLAS H. K. LEE, KATHLEEN W. ROBINSON, NEIL T. M. YEATES, AND MARGARET I. R. SCOTT. (1945). Poultry Husbandry in Hot Climates. Poul. Sci. **24**, 195-207

An interesting article on the reactions of fowls to hot atmospheres and a valuable contribution to the subject of avian physiology, which is in a neglected state.

The animal experiments were conducted in the Southern Hemisphere in air-conditioned room and the duration of each experiment was seven hours, unless the fowl's life was threatened on account of high rectal temperature (113°F.).

Air temperature of 80°F. (dry bulb) is comfortable for the fowl, but beyond this, slight disturbances of bodily functions become obvious. The fowl cannot safely withstand an air temperature of 100°F. for seven hours, unless the relative humidity is less than 75 per cent, while at 105°F. the fowl can withstand only a few hours whatever be the humidity. The authors have observed, that if shade temperatures above 105°F. are likely to be experienced for more than one hour during the day, the well-being of the hens will be upset and death may occur. In Australia it has been observed that if at any time before 1 p.m. the air temperature exceeds 100°F. active precautions are necessary as it is likely to be a hot day and the temperatures remains high until 5 p.m.

Ventilation of poultry houses is helpful in preventing exessive moisture and removing the heated air from houses.

The provision of a constant and adequate water supply, permitting the bird to douse the heap whilst drinking, is stressed.

It has been stated that the level of protein in the diet has nothing to do with heat tolerance.

Hens can become acclimatized to hot environment but in the initial stages the acclimatization may be accompanied by a drop in eggs production.

Six breeds of hens, consisting of both light and heavy, have been studied; Leghorns (white and brown), Minorca, White Wyandotte, Australorp, and Rhode Island Red. Under hot conditions (105°F. with 25 per cent relative humidity) the White Leghorn showed the least rise of temperature and the Brown Leghorn the greatest, while the heavy breeds—Australorp and Rhode Island Red, were intermediate. It has been stated, that generally a hen laying under hot conditions is likely to be affected with heat stroke. In the opinion of the authors, under shade conditions, colour is perhaps immaterial.

In house construction it has been observed that protection from heat can be given by galvanized iron, while washed on the outside, or by covering the roof with creepers of thatch.

The indicatons of heat effects in a hen are: leg weakness, panting and wing drooping, sulking in corners, staggering gait, ultimately leading to collapse. Simple immersion in cold water is a very effective means of saving the bird, but the bird should be placed in a cool airy place to dry out.

(Work along these lines to study the effect of heat upon growth, egg production, and reproduction is called for under Indian conditions, so that the poultry industry may be able to maintain scientific control of breeding and production performance.) (S.G.I.)

O. WILFORD OLSEN (1946). **Hexachlorethane-Bentonite suspension for the removal of the common Liver-fluke, Fasciola hepatica from Sheep.** *Am. J. Vet. Res.* 7,14 : 358-364

AFTER a review of literature on hexachlorethane as a fasciolicide in sheep and cattle, the author describes his experiments with hexachlorethane-bentonite suspension for the removal of *Fasciola hepatica* from sheep. The drug was quite effective and well tolerated by sheep.

Hexachlorethane-bentonite suspension was prepared as an aqueous drench by mixing 500 gm. of commercial hexachlorethane ground to finer than 60-mesh size, 50 gm. of bentonite, ¼ to ½ tea-spoonful of white flour and 750 c.c. of tap water added slowly while stirring. The final mixing was done by passing the wet ingredients through a fine sieve. The suspension was administered orally in a single dose. The results of the treatment were determined by egg-counts, post-mortem examinations, and condition of flocks in which losses were occurring previously.

Of 110 infected sheep given 30 c.c. of suspension (15 gm. of hexachlorethane) each, 104 were found negative for liver-fluke ova after a single treatment. Little change occurred in the egg-count of three animals whose original counts were 16 per gram or less. The remaining three sheep showed only four eggs per gm. as compared with a premedication count of 60 to 112.

Of 20 sheep, examined post-mortem, 13 were treated each with 30 c.c. and seven each with 60 c.c. of the suspension. Necropsy of the former lot, revealed immature flukes in six animals and mature in five, and the mean number per

sheep of immature flukes was 12 ± 29·3 and that of adults was 2·07 ± 5·29. While necropsy of the latter lot showed immature flukes in three and mature flukes in one only and the mean number per sheep of immature flukes was10 ± 13·74 and that of adults was 0·57 ± 1·69. The results indicate that immature flukes are more resistant than mature ones. The author suggests that the treatment of animals should be undertaken during the season when there are fewest immature flukes in the body.

To determine the effect of repeated administration of the drug, it was drenched to one small mature ewe and one yearling ram in daily doses of 30 and 60 c.c. for 12 and four days respectively and was found to be well tolerated by both. However, in a footnote, the author states, that some instances of intoxication and death occurred in one locality following treatment of sheep with hexachlorethane as well as with a standard dose of carbon tetrachloride. (M.A.)

REVIEWS

The Use and Misuse of Shrubs and Trees as Fodder with Tables showing composition and digestibility.

(Published by the Imperial Bureau of Pastures and field Crops Aberystwyth, Imperial Forestry Bureau, Oxford, and Imperial Bureau of Animal Nutrition Aberdeen, June 1947 Re. 0-9-0.)

IN the foreword of this recent publication of the I. A. B., the reader is reminded of the importance of the subject with which it deals by the arresting statement that ' It is humbling fact for grass pasture experts to realize that probably more animals feed on shrubs and trees, or on associations in which shrubs and trees play an important part, than on true grass or grass-legume pastures, short and tall-grass ranges, and steppes '.

A brief and very general description is given of the material, now available for grazing and browzing in vast areas on every continent, except Latin America (already dealt with in Bulletin 36) ; of the land on which it grows ; of the system, or lack of system, used by the graziers ; of observations made by the scientists and of their recommendation for regeneration, preservation and development ; of a descriptive list of the fodder trees and shrubs found in each territory of the importance attached to them locally as animal feed. A valuable Table indicates the chemical composition of the edible parts, at various stages of growth of 486 of these trees and shrubs, and another, the nutritive value of 79 of them.

Throughout the world, there is the same tale of ignorant misuse, if not, actual abuse of this indispensable fodder. Although it is within, only the last two or three decades that the subject has received any considerable scientific study, the authorities in each country apparently have little difficulty in confidentally recommending the action needed to stop deterioration. The lamentable fact remains however, that although one reads of what should be done and what could be done, there is little to be told of what has been done. In this respect, India is, with the United States, one of the countries which can report the results of trials actually made.

One of the most useful parts of the publication, will probably be, found to be the list of references at the end of each section.

[G. W.]

MGIPC—III-1-26—9-2-49—520.

ORIGINAL ARTICLES

INVESTIGATION OF CONTAGIOUS ABORTION IN GOATS WITH SPECIAL REFERENCE TO ISOLATION OF *Brucella Abortus* (BANG) FROM GOATS' MILK*

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(Received for publication on 13 January 1948)

THERE exists a loose natural host specificity amongst the organisms of the genus *Brucella* parasitizing cattle, goats and swine amongst the domestic animals. Of these, *Brucella abortus*, the common causative agent of bovine abortion has been recovered from naturally infected horses, fowls, dogs, sheep, wild dear, wild buffalo and human beings [Huddleson, 1943]. *Br. melctensis*, the organism of goat abortion, has been known to cause 'undulant fever' in man and parasitizes cattle producing abortion in this class of animals. *Br. suis*, which causes abortion in sows, has been isolated from the horse, the fowl, the dog and human beings. Of the two species causing abortion in goats and cows, *Br. melctensis* may naturally infect cows [Parisot, Vidal and Levy, 1932; Giles, Peres and Culty, 1932 and Taylor, Lisbonne and Roman, 1932] the natural host of *Br. abortus*. Very little information is really available regarding the susceptibility of goats to natural infection with *Br. abortus*, though the organism is capable of producing abortion in goats [Doyle, 1939] when introduced artificially.

Positive evidence regarding natural infection of goats with *Br. abortus* emanates from the work of Meurou and Pineau [1937] in France. These authors isolated a strain of *Br. abortus* from the milk and blood of a case of abortion in a goat that had remained for sometime in contact with an aborting heifer. Others, particularly Haddow [1934] in India and Holth [1937] in Norway carried out agglutination tests in large herds of goats and found a considerable number of reactors to *Br. abortus* antigen.

In the present investigation *Br. abortus* was isolated from the milk of goats in a badly infected farm where cases of abortion in goats have been occurring annually since 1929. The finding reported in this paper, therefore, bears some significance in that, firstly, it tends to supplement an already meagre information on natural infectivity of goats to *Br. abortus* and secondly, it represents the only authentic record of the isolation of *Br. abortus* from goats in an infected farm.

Short review of previous work

The following review of work done during the past years has been prepared from the research record files maintained at this Institute. This showed, that most of the work was carried out with materials obtained from the Government Livestock Farm, Hissar, in the East Punjab.

The first outbreak of the disease amongst Beetal goats of the Hissar Farm was reported in 1929. Agglutination test carried out with the sera of some of the aborted goats with the stock antigens of *Br. abortus* and *Br. melctensis* has yielded a negative result. Heart blood and the stomach contents of foetuses and the vaginal swabs of some freshly aborted female goats on cultural examination has yielded a stumpy cocci-like organism which did not agglutinate when put to test against the sera of aborted animals. In this outbreak, as a sequal to abortion, some goats were reported to have died of metritis. The condition was again reported in the following year in which *Br. melctensis* was isolated from the milk of one of the goats. In this outbreak the largest number of abortions occurred during September and November amongst the Jamunapari goats. A large number of sera put to agglutination tests with *Br. abortus* and *Br. meletensis* antigens was found to react positively against one or the other of these antigens. Infective materials from aborted foetuses and vaginal

* Read at the Indian Science Congress, Patna Session 1948.

secretions and placentae from aborted goats on cultural examination did not yield any organism which could be incriminated for goat abortion. Drenching of infective placental emulsion to pregnant goats has caused in one of the goats the expulsion of premature kid from which an organism suspected to be *Vibrio foetus* was isolated. Further study with this organism was not pursued and the results of the investigation was summarized with the conclusion that more than one organism might be the cause of goat abortion on this farm.

In 1933, sera from 175 goats consisting of Toggenburg, Jamunapari, Barbari and local breeds belonging to the Experimental and Research Goat Breeding Farm Etah, United Provinces, were tested against *Br. abortus* and *Br. melctensis* antigens. With the exception of four goats which reacted positively to *Br. abortus* antigen, the rest proved negative to both the antigens. In 1934, out of 54 sera from the same farm, only two reacted positively to *Br. abortus*. In 1935, out of 151 sera tested two proved suspicious and the rest negative to *Br. meletensis* antigen.

The above review indicates that the previous investigations on caprine Brucellosis in India were mainly limited to agglutination test of sera from either known aborted cases or from in-contact animals on a farm where abortions had occurred. Bacteriological investigations carried out with infective materials received from some of the outbreaks did not afford any definite evidence regarding the isolation of *Br. abortus* from aborted goats' tissue or secretions.

Present investigations

The present investigations were carried out amongst goats of the Government Livestock Farm, Hissar, in the East Punjab. The farm comprising about 46,000 acres of land is essentially a cattle breeding farm, but other livestock including goats, sheep, horses, donkeys and camels are also bred though on a limited scale. The animals live under ranch conditions which provide sufficient grazing. Shelter from rains is provided during the monsoon period. Concentrates are fed to draft or working animals or to those in milk or pregnancy, according to requirements.

As stated in the previous section of this paper the existence of Brucellosis amongst the goats of this farm has been proved by agglutination test in 1929. During the subsequent years also the disease had been occurring from time to time, but investigations carried out during these years with the object of determining the type of *Brucella* or any other microorganism involved in these outbreaks had failed to yield any conclusive results. The later efforts since 1937, therefore, were directed towards controlling of abortion rates by complete segregation of aborters and taking hygienic measures. Table I contains the incidence of abortion during 1937-1946.

Table I

The incidence of abortion during 1937-1946.

Year	Percentage of abortion
1937—1938	13.1
1938—1939	0·9
1939—1940	0·9
1940—1941	7·6
1941—1942	2·25
1942—1943	15·1
1943—1944	23·7
1944—1945	not known
1945—1946	1·5

During the year 1937, the incidence of abortion had reached fairly a high figure of 13·1 per cent. and during the subsequent three years the hygienic control measures had brought about considerable improvement. Since the year 1940, it has been the routine practice in the farm to blood test all the goats for Brucellosis and remove the positive reactors separately, thus setting up two herds, e.g. the Brucella-positive and the Brucella-negative herds. The position as revealed by agglutination test from the vear 1940-1946 is given in Table II.

TABLE II.

Agglutination test for the year 1940-1946

Date of test	Number tested	Number positive	Percentage positive
February 1940	66	1	1·5
April 1940	22	..	..
January 1941	24	..	..
July 1943	118	12	10·2
August 1943	197	27	13·7
February 1944	149	1	0·7
August 1944	120	3	2·5
August 1946	226	12	5·3

The two herds policy based on agglutination test seems to have had the effect of lessening the incidence of positive reactors on completion of the third testing of the herd in the year 1941. Two years later, when the herd was augmented by fresh purchases the incidence of reactors had again increased ; in 1945 no tests were made and the following year's test showed some increase over the previous year.

Brucella-negative herd

This herd comprized of 214 goats on the basis of agglutination test carried out by one of us (P. R. N.) in August 1946. Of these, 11 were breeding bucks. Abortions had occurred in two goats 892 and 105 in the last week of July, i.e. about four to five weeks before visiting the farm. Blood serum of these animals were found negative for Brucella infection. Vaginal washing from each were inoculated subcutaneously in 1 c.c. dose to guinea pigs which were destroyed six weeks *post injectio*. Liver and spleen materials were cultured on crystal violet liver infusion-agar plates and incubated aerobically as well as under 10 per cent Co_2 tension at 37°C for seven days. No growth of *Brucella* in any of these were observed.

Another goat 242 aborted on 20th September 1946. Heart blood and stomach contents of a three months old feotus expelled from the goat were sown in tryptose broth culture tubes which were sent to Mukteswar for incubation and isolation of the organism. No pathogenic organism was isolated from these materials. About 1 c.c. of a mixture of the stomach contents and heart blood of the foetus was inoculated simultaneously to a g. pig, but no growth of *Brucella* was observed on crystal violet-liver infusion-agar plates cultured with the spleen and liver materials of the g. pig destroyed six weeks *post injectio*. Milk samples obtained from this goat were cream separated and inoculated in 1·5 c.c. dose to two g. pigs. Another sample preserved in 1 per cent boric acid solution was sent to Mukteswar for cultural examination. No organism of pathogenic significance was isolated either from milk or the liver and spleen of the g. pigs. Blood serum of this goat was tested on the fourth day and three weeks after abortion for Brucellosis and Salmonellosis, but the results were negative for both.

Brucella-positive herd

This herd consisted of 12 adult she-goats, one breeding buck and a few kids. All the adult goats except the buck were found to be positive reactors. Of these, five were pregnant, five had kidded three to four weeks before the farm was visited and two were sterile. No proper breeding history of this herd was available. One of the sterile goats was destroyed for the isolation of *Brucella* organism but no growth occurred on crystal violet-liver infusion-agar plates sown with liver and spleen materials of g. pigs inoculated with saline emulsions of the iliac and supra mammary lymphatic glands of the goat. Vaginal swabs and normal saline washings from the vaginae of goats 26, 685, 753, 918 and 985 which had recently kidded were cultured and inoculated into g. pigs respectively for the isolation of *Brucella* organism. No growths were observed after seven days' incubation of crystal violet-liver infusion-agar plates inoculated with the original swab material and the spleen and liver material of the g. pigs inoculated with the washings.

Examination of milk

About 10 c.c. of milk from each of the above five goats were collected in sterile tubes with aseptic precautions and kept in ice undisturbed for eight hours, 2 c.c. of cream from each sample were inoculated subcutaneously into g.pigs. The g. pig inoculated with the material from goat 26 died on the same day but no cultural examination was made due to highly putrified state of the carcase. Milk samples collected in the same manner and preserved in 10 per cent boric acid solution were also sent to Mukteswar for direct cultural examination, but no organisms were recovered from these samples. The remaining g. pigs were destroyed six weeks *post injectio* and their liver and spleen were cultured on crystal violet-liver infusion-agar plates which were incubated aerobically as well as under 10 per cent Co_2 tension at 37°C. Five days after incubation, plates exposed to Co_2 tention and sown with the liver and spleen materials of g. pigs inoculated with the cream samples from goats 753, 918 and 985 showed colonies similar to *Brucella*. The colonies were picked and subcultured on liver agar slants and grown under 10 per cent Co_2 tension.

Identification of the Brucella *strains isolated*

Each of the three strains was examined as follows:

1. For morphological and staining characters.
2. Against a positive *Br. abortus* serum.
3. For the production of H_2S
4. For growth on Huddleson's dye media
5. For agglutinin-absorption test
6. For pathogenicity to g. pigs.

1. *Morphological and staining characters.* Culture smears were stained by Gram's method and examined. Gram negative, short and slender rods were observed.

2. *Test against a positive* Br. abortus serum. Suspensions of the organisms were made in 1·2 per cent saline which was 0·5 per cent carbolized and adjusted to Brown's opacity tube No. 1. These, together with the standard *Br. abortus* antigen, were put to agglutination test against stock *Br. abortus* anti-serum. Positive agglutination was observed in high dilutions of the serum in all the cases, thus proving that the strains under study belonged to the genus *Brucella*.

3. *H_2S production.* The goat *Brucella* strains along with the known *Br. abortus*, *Br. melitensis* and *Br. suis* strains were subcultured on liver infusion agar slants. Strips of filter paper dipped in 10 per cent lead acetate solution and dried were inserted so that the free ends of the strips would hang inside the culture tubes just above the level of the media. The tubes were then incubated at 37°C (10 per cent Co_2 tension was used wherever necessary). The strips of indicator paper were changed daily and the date and the culture number marked on them. This process was repeated for seven days and on comparison with the known strains the goat strains of *Brucella* had produced

H_2S to the same extent as the known *Br. abortus* strain. The results are shown in the following Table.

TABLE III

Comparison of goats strain Brucella *with* Br. abortus *strain.*

Strains	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Br. abortus (*A1*)	+++	+++	+	±	—	—	—
Br. meletensis (*M1*)	—	—	—	—	—	—	—
Br. suis (*S1*)	+++	+++	+++	+++	++	+	+
Br. isolated from goat 753	+++	+++	+	±	—	—	—
Br. isolated from goat 918	+++	+++	+	±	—	—	—
Br. isolated from goat 985	+++	+++	+	±	—	—	—

4. *Growth on Huddleson's dye media.* Forty eight hours' growth of the three known species of *Brucella* and the goat *Brucella* strains were washed in 8 c.c. of normal saline solution and a loopful of the emulsion from each tube was streaked on tryptose agar plates containing thionin and basic fuchsin separately, as well as on plates without the dyes. The plates were then incubated for four days at 37°C using 10 per cent Co_2 tension wherever necessary. Each day the plates were examined for growths. The goat *Brucella* strains resembled *Br. abortus* in that they were inhibited by thionin but grew equally well on plates containing basic fuchsin and on those without the dyes. The results are tabulated below :

TABLE IV

Growth on the Huddlesons dye media of different Brucella *strain*

Strains	Growth on tryptose agar plate without dye	Growth on tryptose agar plate with basic fuchsin	Growth on tryptose agar plate with thionin
Br. abortus (A1)	+++	+++	—
Br. meletensis (M1)	+++	+++	+++
Br. suis (S1)	+++	—	+++
Br. isolated from goat 753	+++	+++	—
Br. isolated from goat 918	+++	+++	—
Br. isolated from goat 985	+++	+++	—

NOTE.—The dilutions of the dyes in the media are basic fuchsin 1 :90,000 and thionin 1 :60,000.

5. *Agglutinin-absorption test.* Type specific (*abortus* and *meletensis*) sera were prepared by absorbing species-specific sera of a titre of about 1 : 640, with heterogenous organisms. Standard suspensions of the organisms were prepared in the same manner as that for the standard *Brucella* antigens preparation and each of them put to agglutination test against various dilutions of the two type specific-sera. Standard *Br. abortus* and *Br. meletensis* antigens were also put to test in the same manner.

The results tabulated below suggest clearly that goat strains belong to the *abortus* type antigenically.

TABLE V

Results of agglutination absorption test.

Standard antigens prepared from strain	Serum dilutions									
	Br. abortus type serum					*Br. meletensis* type serum				
	1/40	1/80	1/160	1/320	1/640	1/40	1/80	1/160	1/320	1/640
Br. meletensis (M1)	+	—	—	—	—	+++	+++	+++	+++	+
Br. abortus (A1)	+++	+++	+++	+++	+	+	—	—	—	—
Br. isolated from goat 753	+++	+++	+++	+++	+	+	—	—	—	—
Br. isolated from goat 918	+++	+++	+++	+++	+	+	—	—	—	—
Br. isolated from goat 985	+++	+++	+++	+++	+	+	—	—	—	—

6. *Pathogenicity test in g. pigs.* A rough preliminary test for pathogenicity of the goat strains of *Brucella* was made by inoculating subcutaneously 1 c.c. of washed cultures on liver infusion agar slants to g. pigs which were autopsied six weeks *post injectio*. The gross lesions were observed in the spleen, liver and kidneys. The spleen was enlarged 8 to 10 times than the normal and was studded with small well-raised nodules with greyish white centre and haemorrhagic periphery. The liver was congested and slightly enlarged. A few greyish white minute nodules were observed in the cortex of the kidneys under the capsule. *Br. abortus* was isolated in pure culture from these lesions.

DISCUSSION

A survey on Brucellosis amongst the domesticated animals in India, as carried out under the auspices of the Indian Council of Agricultural Research [1940 to 1945] does not contain definite statement regarding the causative organism of this disease in goats. *Br. meletensis* the commonly known organism infecting goats was isolated only on one occasion from a goat in the Punjab, the place of origin of the present strains of *Br. abortus*. Our own review of the disease position in this country with particular reference to the Punjab is compatible with the statement made in this survey in that a number of epidemics or endemics of abortion in goats have been reported from time to time. Our observations, however, are at variance in that in our hands a large percentage of goats in the Hissar farm had proved reactors to agglutination test.

In isolating organisms from mor-bid materials in the field in India, climatic factors have often been stressed to account for failures in obtaining pure cultures or no growths at all, the latter particularly in such affections as abortion which occurs without any previous warning, and when bacteriological equipments and personnel are late to arrive. These difficulties in our work were considerably obviated by our knowledge of the kidding season in the Hissar farm, moving equipment (including g.pigs) and personnel to the farm and staying there for a month during which period abortions or normal kiddings were expected.

Vaginal swabs and washings from the reacting goats which had normally kidded yielded no organism either on direct examination or *via* g. pigs. This might be explained on the basis of information already available [Huddleson, 1943] that even in the most acutely infected goats when the height of infection is passed, elimination becomes sporadic, and that a single negative culture can hardly

be regarded as informative. Further, it is probable that during the sixth week after kidding when the vaginal materials were collected, the vaginae might have ceased to function as the channel of elimination. Our success in isolating the organism from the milk would only indicate that during the period after kidding when the samples were collected the organisms had localized in the udder and were being eliminated through the milk. Where the blood serum response is positive in a high titre, the evidence of infection by *Brucella* organisms may be considered as irrefutable, although it might not be possible to demonstrate in every such case the organism by cultural examination.

In view of the isolation of *Br. abortus* from milk samples of goats of a farm subjected to frequent epidemics or endemics of goat abortion, the question arises as to the possible relationship of the infection to cattle of the farm and *vice versa*. Although cattle abortion had been occuring in this farm, it never assumed the same seriousness as amongst the goats and as such, no investigation on the incidence of the disease amongst the bovines was called for. From a few trickles of abortion that have been reported, the organisms isolated were reported to conform to *Br. abortus-meletensis* type. The goats from which *Br. abortus* was isolated in of the present investigation were purchased from some adjoining district and being kept separate from the bovine herd, chances of infection being acquired by them from cattle could, therefore, be considered remote. Strict isolation of these two herds may also account for the disease being localized amongst the goats only.

SUMMARY

1. Brucellosis amongst goats at the Government Livestock Farm, Hissar was investigated by blood serum agglutination test and by cultural examination and g. pig inoculation of infective materials including milk of some normally kidded goats.

2. From a few serum-positive goats, *Br. abortus* (Bang) was isolated by milk samples inoculated into g. pigs. The essential criteria used in the differentiation of the genus *Brucella* were tested in the present work of typing this organism.

ACKNOWLEDGEMENTS

The authors wish to place on record their gratitude to Dr. F. C. Minett, Director, Indian Veterinary Research Institute for affording facilities in the execution of this work, including a full use of the research record files of the Institute for the period 1929-35. Thanks are also due to the Superintendent, Livestock Farm, Hissar, for making necessary arrangements and for allowing to collect information from the records and files of the farm.

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INFECTIVITY BY CONTACT OF RINDERPEST VIRUS, INCLUDING GOAT-ADAPTED VIRUS, FOR CATTLE AND GOATS*

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(Received for publication on 3 February 1948)
(With four text figures)

EDWARDS [1927] succeeded in implanting virulent rinderpest bull virus in goats by passing it through the foetal membranes of pregnant animals. In this way, a goat-adapted rinderpest virus was elaborated which, while retaining its specific pathogenicity for goats, was of value for protecting cattle and which is in use for this purpose in India up to the present time. This weakened virus, produces in Indian cattle only a mild thermal reaction but the immunity is serviceable and lasting, and under experimental conditions at Mukteswar has been proved to endure for some ten years. After extensive use of this product for nearly twenty years, both in free areas and in the face of outbreaks, almost no adverse comment has arisen as to its value and safety. On the other hand, imported cattle, water buffaloes, cattle of pure-bred progeny, and native cattle with an admixture of foreign blood tend to show a more severe thermal reaction and other ill effects. To control such undesirable reactions it is well known to be sufficient to inject at the same time a small quantity of rinderpest immune serum, say 10 to 40 c.c. per 100 lb. body weight. This serum-simultaneous method using goat-virus has also been adopted with satisfactory results, both under laboratory and field conditions [Bawa, 1940] for protecting sheep and goats against rinderpest. During the past twenty years the general observation has been made that the use of goat-virus produces centres of infection neither in native cattle nor in more susceptible cattle. Experiments in the same connection have been made, both at Mukteswar and in the field, to determine whether cattle undergoing vaccination with the modified virus are infectious for healthy cattle and goats by contact. As a result, it seems evident that under what may be called ordinary conditions of contact transmission does not occur. However, it still remained to be ascertained whether transmission might occur under conditions, some of them admittedly rather artificial, which would enhance the chances of transfer. The results of experiments in this light are reported here.

EXPERIMENTAL

The strains of virus used were (i) a virulent bull virus, designated Line 'E'. This virus is highly pathogenic for hill bulls and causes a mortality of 95 per cent. (ii) a goat-adapted virus, designated Line 'W', which is maintained at this Institute for supplying goat spleen tissue to the field for immunization work. Representative temperature charts of these viruses in bulls and goats are given in Figs. 1 to 4. The animals used in the experiments were goats and Kumauni hilll bulls. The latter according to Edwards, are 18 times as susceptible to rinderpest as plains cattle. The goats were brought from the plains and the goat-modified virus has a specific pathogenicity for these animals.

To represent various degrees of contact that are possible under ordinary field conditions, experiments were made as follows:

(i) In a cattle shed. This was of the double row type, 50×20 feet, in which the animals are tied facing away from one another. At each end there are two doorways, between which runs a central passage, three feet wide. The house has no separate feeding passages and is divided into stalls, six feet wide. For ventilation there are skylights in the roof and three windows in each of the long walls. In this experiment two animals were tied in the one stall, the windows were always closed and doors opened except at night.

(ii) In a chappar, where still closer contact between healthy and infected was possible.

This was really a small room, 10 feet square, and about large enough to accommodate two hill

*Paper read at the Indian Science Congress 1948.

bulls and two goats or one hill bull and four goats. Ventilation is provided by a window and the animals are let loose inside and all the animals feed and drink from the same utensils.

(iii) In a trevis—according to the method recently used by Idnani [1944]—so that the two animals one infected and one healthy, face each other at a distance which can be varied at will, the apparatus being covered with a thick tarpaulin so that the healthy one inhaled the expired air of the infected one.

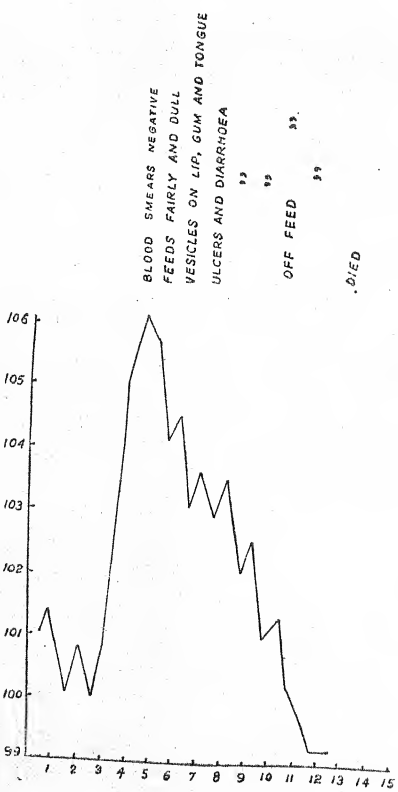

FIG. I. Line 'E' virus in hill-bull.

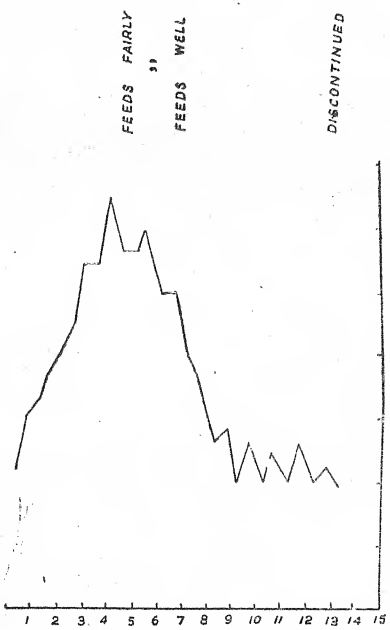

FIG. 2. Line 'E' virus in goat.

EXPERIMENTS WITH GOAT-ADAPTED VIRUS

1. *Shed contact*

(*a*) *Goat as donor.* The goat virus is maintained by series of passages in goats, blood being taken at the height of fever usually on the fourth day, and sub-inoculated into two other goats. In the stall where the virus producers are accommodated, it was arranged that two healthy goats and one bull should be kept and that these animals should remain in contact with not less than ten consecutive batches of virus producers. The temperatures of the experimental animals were not taken so as to avoid any chance of mechanical transmission. Three weeks later the two goats and the bull were proved to be susceptible to rinderpest on being tested with goat virus and bull virus respectively.

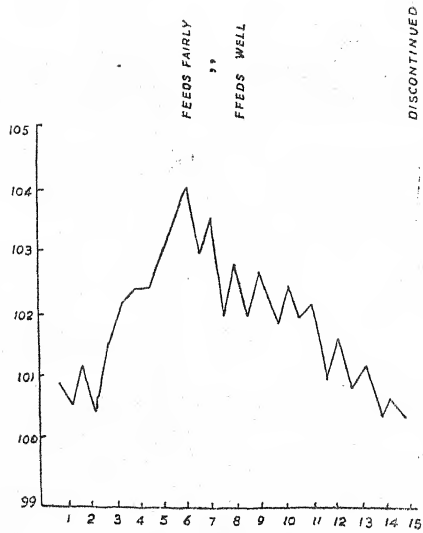

FIG. 3. Line 'W' in hill-bull.

(*b*) *Bull as donor.* A healthy bull was inoculated with the goat-adapted virus and accommodated in a stall of the shed described above. In the same stall were tied two goats and one hill bull so that there was appreciable contact between them. Although the inoculated bull reacted, the in-contact animals showed no rise in temperature and were later proved susceptible to rinderpest.

2. *Chappar contact*

In the next attempt a chappar was used.

(*a*) *Goat as donor.* In this experiment two goats inoculated with goat virus were placed in company of two healthy goats and one hill bull. The inoculated goats died nine and ten days after inoculation. The in-contact goats and bull showed no signs of infection. After three weeks they were tested with line 'W' and line 'E' virus respectively, and shown to be still susceptible.

(*b*) *Bull as donor.* A hill bull was inoculated with goat virus, and two goats and one bull were allowed to remain in contact for a period of three weeks. Although the inoculated bull showed a thermal reaction, the in-contact animals showed no signs of infection and all proved susceptible to rinderpest.

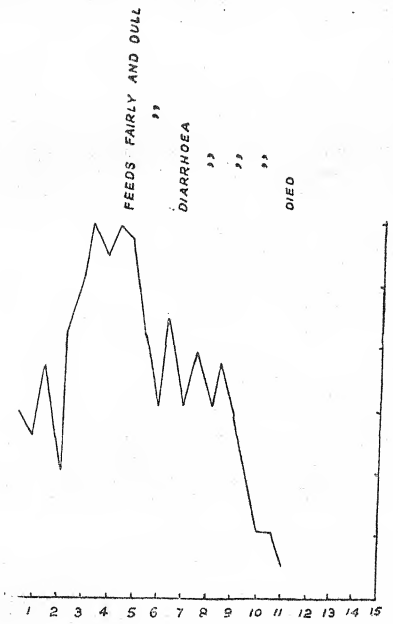

FIG. 4. Line 'W' virus in goats.

3. *Trevis contact*

(*a*) *Goat as donor.* A goat was inoculated with goat virus. On the third, fourth, fifth, sixth and seventh days after inoculation, a healthy goat was exposed for six hours at a distance of three feet from it. Seven days after the last exposure the animal showed a thermal reaction (104·5°F.), and when tested three weeks later, proved to be immune. In another experiment a goat was inoculated with goat virus. On the third, fourth, fifth, sixth and seventh days after inoculation a hill bull was exposed, for six hours and at three feet distance on each of these days. The goat died on the ninth day, but the bull remained unaffected and was later proved susceptible to rinderpest.

(*b*) *Bull as donor.* Idnani [1944] has proved by the above method that transmission of rinderpest does not take place between a bull inoculated with goat virus and a healthy bull. To find out if by this method, a healthy goat will contract the disease from a bull inoculated with goat virus, a healthy goat was exposed to an infected bull daily from the third to seventh days for a period of six hours at a distance of three feet. The experimental animal remained unaffected and later proved susceptible.

EXPERIMENTS WITH VIRULENT BULL VIRUS

To ascertain the infectivity of a more virulent rinderpest virus by contact from goat to bull, from goat to goat or from bull to goat, a set of experiments was carried out on the lines of work done with goat virus.

1. *Shed contact*

(*a*) *Goat as donor.* Two goats were inoculated with line 'E' virus. Two goats and one hill bull were placed in contact in the same stall. Although the inoculated animals showed a thermal reaction, the in-contact animals remained unaffected and three weeks later proved susceptible on test.

(*b*) *Bull as donor.* Two goats and one bull were in contact in the same stall with one hill bull inoculated with line 'E' virus. The inoculated animal showed typical reaction of rinderpest and died on the 11th day. The in-contact bull contracted the disease and later died of it, whereas the in-contact goats showed no reaction and were susceptible to rinderpest on test.

2. *Chappar contact*

(*a*) *Goat as donor.* Two goats were inoculated with line 'E' virus and kept with two healthy goats and one hill bull. Both inoculated goats showed a thermal reaction and one of them died on the 12th day. All the in-contact animals showed a thermal reaction from the seventh day. The bull died of rinderpest on the 14th day, one goat died on the 11th day and the other goat survived.

(*b*) *Bull as donor.* Two goats and one hill bull were allowed to remain in contact with one hill bull inoculated with line 'E' virus. The inoculated animal showed typical reactions of rinderpest and died on the 11th day. But the in-contact goats did not contract the infection and later proved susceptible on test with goat virus. The in-contact bull contracted the disease and died.

3. *Trevis contact*

(*a*) *Goat as donor.* A goat inoculated with line 'E' virus was exposed to a healthy goat for six hours daily from the third to seventh day, separated by a distance of three feet. In another experiment under similar conditions a bull was exposed. The experimental goat contracted the disease and died. The bull showed no signs of infection and later proved susceptible on test.

(*b*) *Bull as donor.* Experiments done by Idnani have proved that transmission of rinderpest by expired air from an infected bull to a healthy one is possible. To ascertain if a goat would contract the infection under similar conditions, a bull was inoculated with line 'E' virus and a goat exposed to it from the third to seventh day daily for six hours, the distance between the animals being three feet. The experimental animal remained unaffected and after three weeks proved susceptible to rinderpest.

In the tabular summary, the results of transmission experiments described in this paper using the goat-adapted virus (W) and the virulent virus (E) are as follows :

TABLE I

Results of transmission experiments

Strain of virus	Animal inoculated	Animal exposed	Results		
			Shed	Chappar	Trevis
Line 'W'	Goat	Goat	—	—	+
		Bull	—	—	—
	Bull	Goat	—	—	—
		Bull	—	—	—*
Line 'E'	Goat	Goat	—	+	+
		Bull	—	+	—
	Bull	Goat	—	—	—
		Bull	+	+	+*

* Experiment after Idnani, (1944).
† Typical symptoms and death.
— No reaction Still susceptible to rinderpest.

DISCUSSION

Pfaff [1938] at Insein found evidence of the spread by contact of goat virus to buffaloes but not to cattle. However, further spread from buffaloes so infected with goat virus was not seen. In Pfaff's experiments ten Kpaukpu cattle were placed in a small loose box and five of them were given goat virus. All the inoculated cattle reacted to the virus and they were kept in contact with the tested cattle for 30 days. The latter showed no rise in temperature and later reacted typically to a subcutaneous injection of goat virus. Pfaff observes that, although no controlled test was made, it was noticed that there was evidence of the spread of the disease to uninoculated buffaloes by contact with buffaloes inoculated with goat virus. The experiments of Cornell and Oonywongse [1941] support the observations of Pfaff. Waddington [1945] has carried out some experiments to determine whether cattle vaccinated with goat virus are infective for other cattle. He observed, that during the reaction to the vaccination the nasal secretions were infective to susceptible cattle by inoculation but not by natural exposure. He also found that the faeces of reacting animals are non-infective by inoculation. He therefore, expresses the view that the possibility of cattle undergoing vaccination transmitting the disease to healthy susceptibles can be disregarded. Additional evidence in support of this view is available from the experience of different observers in India [Shahi, 1933-34 ; Naik, 1946].

Mohamed [1947], in his observations on the use of goat-adapted rinderpest virus in Egypt states that the goat strain was capable of establishing itself in the field and thus create centres of permanent infection. Mohamad's observations have been commented by Minnet and Seetharaman [1947]. From the vast experience accumulated in India, it is rather difficult to believe this. Crawford [1947] from his experience in Ceylon observes that for some reason rinderpest, which had been unknown in goats imported from India into Ceylon for some years was later found to be present in severe form in many batches of imported goats, and that before long outbreaks of rinderpest appeared in Ceylon amongst buffaloes and cattle kept in the vicinity of the imported goats. Crawford believes that the

use of goat virus may have been responsible for establishing a certain form of epizootic in goats and that this in turn may have been responsible for the spread of a form of rinderpest in cattle. The results of the transmission experiments described in this article, with both the virulent and the goat-adapted rinderpest virus, suggest, however, that when epizootics occur in goats it is most unlikely that they originate from the use of goat virus vaccine. It is well known that outbreaks of rinderpest do occur in goats, but in the light of all the evidence one can only conclude that they are due to the natural virus, probably from cattle. How frequently such outbreaks actually occur in sheep and goats cannot be stated, because the diagnosis of rinderpest in sheep and goats, apart from laboratory procedures, is rather difficult, the symptoms according to Orr [1945] being not unlike those seen in other common diseases in these animals. The possibility of rinderpest virus from natural outbreaks in sheep and goats producing rinderpest in cattle is one which requires investigation. The results of the present experiment suggest that, in general the chances of transmission will depend on the nature of the virus, its virulence and environmental conditions in which goats and cattle happen to be accommodated. In any case, an early diagnosis of rinderpest in goats would be desirable, so that opportunity for its dissemination may be impeded.

Summary

1. The possibility of goats or cattle undergoing vaccination with the goat-modified rinderpest virus spreading the infection to goats or cattle kept in the same shed has been tested. The experiments have given negative results. Under ordinary field conditions, therefore, there appears to be no danger of spread of the disease occurring through this cause.

2. Under artificial conditions of very close contact, as when healthy animals are compelled to breathe the expired air of infected ones, transmission is possible. Important factors here are the virulence of the virus and the degree of exposure in terms of time and distance between the animals.

Acknowledgement

My thanks are due to Dr F. C. Minett for advice and for assistance in writing this paper.

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NASAL CARRIERS IN BOVINE PASTEURELLOSIS

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(Received for publication on 23 December 1947)

THE older view that in nature Pasteurellosis results from the ingestion of *Pasteurella-septica* leading a saprophytic existence in the outer world is no longer tenable. To explain the spread of the disease transmission by vectors had been postulated. Thus Magnusson [1914] suggested that some sucking insect was responsible for a large epidemic which occurred among reindeer in Sweden. Daubney, Hudson and Roberts [1934] adduced some evidence that the disease in cattle could be carried by the flea, *Ctenocephalus-felis-bouche*. This was supported by Mehra [1941-1943] who succeeded in transmitting the disease in rabbits and in large animals through the agency of the same flea. This matter has been investigated by Sapre [1945] with some thoroughness. Using a highly virulent strain of *Pasteurella* he has found that while fleas can transmit the disease with ease in rabbits, such transmission failed or sometimes occurred only with the greatest difficulty in young water buffaloes which are highly susceptible in nature. At the present time it is considered with much justification that in all probability respiratory carriers are responsible for maintaining and spreading pasteurellosis in cattle or as it is still termed, haemorrhagic septicaemia. The same is true of swine [Uhlenhuth and Haendel 1913]. In the case of rabbits, Dekruif [1921, 1922a, b, 1923] observed *Pasteurella* organisms in the nose of a large proportion of apparently normal animals living in association with rabbits suffering from snuffles. Webster [1924a, b] found that preceding an outbreak of rabbits Pasteurallosis there was a rise in the normal carrier rate and that the susceptibility of rabbits varried greatly, some being highly resistant, others succumbing with ease. With fowls, Pritchett, Beaudette and Hughes [1930], showed that 11 per cent. of healthy birds acted as carriers and that it was from such birds that the organisms spread and gave rise during the winter months to the various forms of infections viz., carriers, localized upper respiratory disease and typical fowl cholera. Hughes and Pritchett [1930] showed that *Past. septica* was incapable of inciting fowl cholera when introduced directly into the alimentary tract, but could produce the typical disease when administered into the upper respiratory passages. Jorgenson [1925] found that in cattle a considerable proportion of normal animals (about 15 per cent) carried *Past. septica* in the upper respiratory tract. All such observations suggest that natural infection occurs through the nose and is dependant on the presence of carriers.

The present work was undertaken in order to ascertain the proportion of *Pasteurella* carriers in selected groups of cattle and buffaloes. In all, the nasal passages of 300 animals were swabbed, viz., 100 slaughtered animals at the abattoir and 200 live animals in areas in the United Provinces where Pasteurellosis prevails. The animals examined were mainly young buffaloes. Of the dead animals, 7 per cent were found to be *pasteurella* carriers while the carrier percentages among live animals were :

Area	No. examined	Carriers per cent
1	60	5·0
2	60	3·3
3	80	3·75

EXPERIMENTAL

The swabs were made of copper wire 46 cms. long with a cotton wool binding at one end. This was placed within a shorter rubber tube of 0·6 to 1·0 cm. internal diameter, so that the wool binding lay near one end. Both ends of the rubber tube were then plugged and the whole outfit sterilized. For swabbing the nasal chamber, wet swabs soaked either in sterile normal saline or in sterile broth

were used. Dry swabs did not give satisfactory results. In taking the swab, the wool plugged rubber tube was passed into the nostril through the nares in the live animals and through the oesophagus or after splitting the head in the dead one, the plug pushed out, the mucous membrane rubbed, the wire pushed back into the lumen of the rubber tube before withdrawing the whole outfit from the nose.

Swabs taken from animals at long distances from the laboratory were emulsified in broth at the spot, material taken to the laboratory, incubated for six hours and then plated out. Swabs taken from animals near the laboratory were directly streaked either on blood agar or on serum agar plates which were previously partially dried in the incubator. The plates were then incubated for twenty-four hours when they were searched for colonies like those of *Past. septica* viz., round, low convex with smooth glistening surface and entire edge. Among other organisms often met with on plates were staphylococci, gram-negative diplococci, haemolytic and non-haemolytic streptococci. Suspicious colonies were fished to blood agar slants for further examination. Any organisms which were then found morphologically similar to *Pasteurella* were further studied for fermentation and other characters. Tests were made in 1 per cent dextrose, lactose, maltose, sucrose, mannite and salicine, also for indole and hydrogen-sulphide production, in litmus milk, for gelatin liquifaction, V. P. and M. R. reactions. Cultures conforming to *Pasteurella* were retained for agglutination and pathogenicity tests.

Results

In Table I are shown the reactions of 15 *Pasteurella* strains isolated from dead and living animals.

TABLE I

Reactions of different Pasteurella *Strains*

Ser. No.	Species of animals from which isolated	Source	Sugar fermentation reactions					
			Dextrose	Lactose	Maltose	Sucrose	Mannite	Salicine
1	Cow heifer	Abbattoir	A	—	—	A	A	—
2	Buffalo heifer	Do.	A	—	—	A	A	—
3	Buffall cow	Do.	A	—	—	A	A	—
4	Buffalo bull	Do.	A	—	—	A	A	—
5	Buffalo heifer	Do.	A	—	—	A	A	—
6	Buffalo bull	Do.	A	—	A	A	A	—
7	Buffalo calf	Do.	A	—	—	A	A	—
8	Buffalo bull	Live animal	A	—	—	A	A	—
9	Buffalo bull	Do.	A	—	—	A	A	—
10	Buffalo bull	Do.	A	—	—	A	A	—
11	Buffalo cow	Do.	A	—	—	A	A	—
12	Buffalo bull	Do.	A	—	—	A	A	—
13	Cow heifer	Do.	A	—	A	A	A	—
14	Buffalo calf	Do.	A	—	A	A	A	—
15	Buffalo heifer	Do.	A	—	—	A	A	—

A = Acid, no gas, — = Negative.

All the above strains when subjected to M. R., V. P., litmus milk and gelatin liquifaction reactions proved negative, but they all produced indol and hydrogen sulphide.

Agglutination

The serum dilutions ranged from 1 : 50 to 1 : 3200 and some times higher. Tubes were placed in water bath at 55°C for 2-4 hours or in dry incubator at 37°C for 24 hours. A positive test serum was prepared by injecting rabbits at 4-7 days intervals with increasing amounts of 0·4 per cent formalized culture of highly virulent typical *Past. septica*. Seven injections were given, animals were bled seven days after the last injection and serum was preserved with 0·5 per cent phenol. Antigens were prepared from the 15 strains described in Table I by washing off 24 hours agar cultures and diluting to a density between 1 and 2 (Brown's opacity standard).

An experiment with the 15 strains showed that where agglutination occurred, there was no difference in the titre between live and dead *Pasteurella* antigens. Hence for routine agglutination tests, heat killed antigens preserved with 0·25 per cent chloroform have been used. It was also noted that four of the 15 strains, Nos. 4, 7, 8 and 9 failed to show any agglutination, but nearly all the rest reached a titre of 3200 to 6400. This result was not unexpected in view of the previous work. Cornelius [1929] working with 26 *Pasteurella* strains showed that these varried widely in agglutinibility and that not infrequently strains would become temporarily inagglutinable. He found that infection by a particular serological type was not confined to a particular species of animals. He fitted 17 of the strains into four groups but there was no relationship between serological grouping and the animal species of origion. Yusef [1935] classified 14 out of 21 strains into three serological groups.

Pathogenicity

Tests were made in rabbits by injecting intramuscularly 0·0001 c.c. of 18 hours broth culture. The rabbits were of roughly the same weight and were kept under observation for a week. The results are shown in Table II.

TABLE II

Pathogenicity of strains

Strain No.	Death (d) or Survival(s)	Time (hours) from inoculation to death	Remarks
1	(d)	43	
2	(d)	44	
3	(d)	108	
4	(s)	..	
5	(d)	36	
6	(d)	95·5	
7	(d)	26·5	
8	(s)	..	
9	(s)	..	Died due to some other cause
10	(d)	108	
11	(d)	36	
12	(d)	96	
13	(d)	44	
14	(d)	36	
15	(d)	93·5	

The results show that 12 of the 15 strains were virulent for rabbits although they were not of standard virulence. *Pasteurella* was recovered from the heart blood and internal organs of rabbits that succumbed.

Discussion

The proportion of carriers detected in this work probably greatly under estimates the incidence in nature. For one reason the nasal passages in bovines are very long and it is very difficult to reach their remote parts so that in many animals swabbing is incomplete or otherwise unsatisfactory. Secondly the nasal mucous membrane is very sensitive and the irritation often causes much struggling. Correspondingly the carrier incidence determined in animals after death was somewhat higher viz., 7 per cent compared with 4 per cent in living ones.

Summary

1. Fifteen strains of *Past. septica* were isolated from nasal swabs taken from 300 cattle, mostly young buffaloes. One hundred were from freshly slaughtered animals at the abattoir while the remaining were from live cattle in regions where Pasteurellosis is not uncommon. Among the dead animals the carrier incidence was 7 per cent and in the living one 3·5 per cent. These percentages are probably under-estimates.

2. The strains were similar in being non-motile, non-gas-producing, fermenting dextrose, sucrose and mannite but not fermenting lactose and salicine, and producing indole and hydrogen-sulphide.

3. Four of the 15 strains were not agglutinated by serum prepared with a standard laboratory strain of high virulence. Twelve of the 15 were pathogenic for rabbits.

Acknowledgements

The author wishes to thank Dr F. C. Minett for his direction and assistance in writing the paper. He is also obliged to Messrs L. Shai and J. R. Haddow for helpful criticism and guidance.

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UTILIZATION OF BY-PRODUCTS OF STARCH MANUFACTURE FROM MAIZE AS CATTLE FEEDS

I. THE NUTRITIVE VALUE OF MAIZE HUSK

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(RECEIVED FOR PUBLICATION ON 25 NOVEMBER 1947)

OWING to the increasing demand in recent years for industrial starch, a number of factories in different parts of the country are manufacturing it from maize, with maize-husk or bran and maize-oil cake as by-products. In the United Kingdom and United States of America these by-products are used for stock feeding. The starch manufacture in this country should thus yield two valuable cattle feeds.

Through the courtesy of Messrs Govan Bros., Rampur (United Provinces) one lot, each of maize-husk and maize-oil cake were received for investigations into their nutritive value.

Origin of the by-product

It is understood from the manufacturers that miaze-husk is obtained in the course of starch manufacture as follows :

The maize, as received, is cleaned in a maize cleaner. The grain is then softened by soaking in water slightly acidified with sulphurous acid. The softening requires several days after which the maize kernels are torn apart in crushing mills so as to liberate the germs. The material is then mixed with water and passed into tanks where the germs, which owing to their oil content are lighter, rise to the surface and are removed. The residue is finely ground and strained in stages through silk sieves there by separating husk from the starchmilk. The husk is then pre-dried through a roller mill and thereafter dried by steam driers and finally bagged.

Chemical composition of maize husk

In Table I is shown the chemical compositions of maize husk, corn bran—a by-product of maize similarly obtained in America [Morrison, 1937] and brans from other cereals, such as, wheat and rice [Sen, 1946]. Though similarly produced the Indian by-product (maize-husk or bran) in some constituents differs significantly from the American stuff. It is poorer in crude protein, and markedly so in ether extract but richer in both crude fibre and nitrogen free extract. The Indian by-product is markedly deficient in phosphorus, while the American stuff is extremely poor in calcium.

Table I

Percentage composition of maize-husk and allied by-product

—	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Total carbo-hydrates	Total calcium	ash	Phosphorus
Maize husk . .	8·12	1·52	15·67	72·50	88·17	2·19	0·31	0·09
Corn bran . .	9·80	6·40	9·80	61·80	71·60	2·30	0·03	0·27
Wheat „ (Pusa)	11·39	1·73	16·62	60·36	76·98	—	—	—
Rice „ (Hosur)	10·40	12·98	14·69	39·85	54·54	—	—	—

A comparative study of the composition of maize-husk and wheat and rice bran shows that the husk is poorer in crude protein than the brans. It's ether extract content is about the same as of

wheat bran but much lower than that of rice-bran. The husk contains nearly the same amount of crude fibre as the brans but is significantly richer in nitrogen free extract, being twice as rich as rice-bran. The figures for calcium and phosphorus in the two samples of brans are not available, but from data of other samples [Sen, 1946] it is apparent that, unlike the husk, the two brans are rich sources of phosphorus, though their calcium content is definitely poorer than that of the husk.

Metabolism of maize-husk

For reasons stated later, this investigation had to be carried out in two experiments :

EXPERIMENT I

The object was to study the maximum quantity of maize-husk taken by cattle when given free access to it, and with maximum consumption of husk and wheat-bhoosa fed *ad lib* to determine (*a*) the digestibility coefficient of the whole ration as well as of maize-husk and (*b*) nitrogen calcium and phosphorus balances. Four adult healthy kumaoni bullocks of average live weight of 300 lb. were selected for experiment. In a preliminary trial, an attempt was made to assess the maximum quantity of the husk the animals would consume. They seemed to relish the stuff and the daily consumption varied between 4 to 5 lb. per animal. In the course of about a week, however, consumption gradually fell. The method of feeding was then changed by mixing finely ground common salt with the husk. This improved the consumption and just before the experimental trial it was stabilized at about 3·5 lb. During the trial each animal was given 4 lb. of the husk mixed with one ounce of common salt, the residue left over was weighed and recorded. After the consumption of the husk, which was placed before the animals at 9.30 a.m. and the residue removed after about 3 hours, weighed quantity of wheat-bhoosa in excess of the actual requirement was offered. A record was also maintained of the residue left of the wheat-bhoosa. This ration was fed for a preparatory period of 15 days, after which 24 hourly collections were made of excreta for 10 days to determine the digestibility and balances of nitrogen, calcium and phosphorus. During the feeding trial, the animals were frequently weighed. The weight at the end of the trial revealed that all the animals suffered a mean loss of weight of 7 lb.

Food consumption. In Table II is given the consumption on dry basis of individual items of the ration per head per day, as also the total dry matter consumption per 100 lb. live weight. The data in Table II show that the average daily consumption of maize husk in three out of four animals was a little over 1400 gms. in one animal it was slightly less than 1200 gms. The daily consumption, when averaged for the four animals works out at 1354·6 gms. maize husk per animal per day. This high intake of husk supplemented simply by common salt seemed to have seriously affected the consumption of wheat-bhoosa which was as low as 66·8 gms. per animal per day. The lowered intake of roughage was reflected in the total dry matter consumption per 100 lb. live weight. Considering that the average capacity of consumption of dry matter, under maintenance condition of an adult, should ordinarily approximate 900 gms. per 100 lb. live weight, the average intake of 672·8 gms. dry matter indicates that the actual consumption is about 25 per cent below the expected normal. It would be of interest to trace the reasons for this depression in intake when the feeds were offered freely. One of the possible factors limiting the optimum intake may be the physical effect of swelling which maize-husk may undergo in the rumen. But perhaps a more potent factor is the unusually low phosphorus content of the husk. It is well known that one of the earliest physiological disturbances produced by phosphorus deficiency is depression in appetite [Tjeiler and Green, 1932]. The data presented later seem to justify this contention.

Results of metabolism trials

In Tables III and IV are detailed the composition of the feeding stuff used in the experiment and the consumption and excretion of various nutrients and their digestibility coefficients. The balances of nitrogen, calcium and phosphorus are shown in Table V. The significance of these data on metabolism will be discussed later.

TABLE II

Food consumption per day (on dry basis) in grams. Experiment 1

	Wheat bhoosa	Maize husk	Total consumption	Consumption per 100 lb. live weight
Bullock No. 1	541·3	1182·2	1723·5	590·4
„ No. 2	756·8	1414·6	2171·4	705·1
„ No. 3	688·4	1414·6	2103·0	691·8
„ No. 4	676·6	1407·0	2083·6	704·0
Average	665·8	1354·6	2020·4	672·8

TABLE III

Percentage composition of feeding stuffs (on dry basis). Experiment 1

	Organic matter	Crude protein	Ether extract	Crude fibre	N.-free extract	Total carbo-hydrates	Total ash	Calcium	Phos-phorus
Wheat-bhoosa	86·23	2·39	0·99	40·21	42·64	82·05	13·77	0·34	0·07
Maize-husk	97·81	3·12	1·52	15·67	72·50	88·17	2·19	0·31	0·09

TABLE IV

Digestibility coefficient of whole ration and maize-husk. Experiment 1

	Crude protein gm.	Ether extract gm.	Crude fibre gm.	N. free extract gm.	Total carbohy-drates gm.
	Bullock No. 1				
Consumed from—					
Wheat-bhoosa	12·94	5·36	217·67	230·84	448·51
Maize-husk	95·95	17·91	185·30	857·10	1,042·40
Total	108·89	23·27	402·97	1087·94	1490·91
Voided in faeces	63·39	15·49	145·05	295·38	440·43
Total digested	45·50	7·78	257·92	792·56	1050·48
Digestibility coefficient of the whole ration	42	33	64	73	72
Digested from—					
Wheat-bhoosa	—	1·93	132·78	122·34	255·12
Maize-husk	45·50	5·85	125·14	670·22	795·36
Digestibility coefficient of maize husk	47	33	68	78	76

TABLE IV—*contd.*

	Crude protein gm.	Ether extract gm.	Crude fibre gm.	N. free extract gm.	Total carbohydrates gm.
		Bullock No. 2			
Consumed from—					
Wheat-bhoosa	18·09	7·49	304·33	322·72	627·05
Maize-husk	114·79	21·43	221·72	1025·60	1247·32
Total	132·88	28·92	526·05	1348·32	1874·37
Voided in faeces	75·27	21·33	197·13	383·44	580·57
Total digested	57·61	7·59	328·92	964·88	1293·80
Digestibility coefficient of the whole ration	43	26	63	72	69
Digested from—					
Wheat-bhoosa	—	2·70	185·65	171·04	356·69
Maize-husk	57·61	4·89	143·37	793·84	937·11
Digestibility coefficient of maize-husk	50½	23	64	77	75
		Bullock No. 3			
Consumed from—					
Wheat-bhoosa	16·45	6·81	276·72	293·56	570·28
Maize-husk	114·79	21·43	221·72	1025·60	1247·32
Total	131·24	28·24	498·44	1319·16	1817·60
Voided in faeces	81·59	20·55	209·02	453·03	662·65
Total digested	49·65	7·69	289·42	865·53	1154·95
Digestibility coefficient of the whole ration	38	27	58	66	64
Digested from—					
Wheat-bhoosa	—	2·45	168·85	155·59	324·44
Maize-husk	49·65	5·24	120·57	709·94	830·51
Digestibility coefficient of maize-husk	43	25	54	69	67
		Bullock No. 4			
Consumed from—					
Wheat-bhoosa	16·18	6·70	272·71	288·53	561·24
Maize-husk	114·18	21·31	220·52	1020·00	1240·52
Total	130·36	28·01	493·23	1308·53	1801·76
Voided in faeces	75·77	20·89	201·91	416·64	618·55
Total, digested	54·59	7·12	291·32	891·89	1183·21
Digestibility coefficient of the whole ration	42	25	59	68	66
Digested from—					
Wheat-bhoosa	—	2·42	166·35	152·93	319·28
Maize-husk	54·59	4·70	124·97	738·96	863·93
Digestibility coefficient of maize-husk	48	22	57	73	70

TABLE V

Nitrogen balance. Experiment 1

No. of Animal	Intake gm.	Out put		Total gm.	Balance
		Faeces gm.	Urine gm.		
		Nitrogen balance			
1	17·45	10·15	10·14	20·29	—2·84
2	21·30	12·04	10·38	22·42	—1·12
3	21·04	13·02	10·10	23·12	—2·08
4	20·90	12·12	10·06	21·18	—1·28
Average	20·17	11·83	10·17	22·00	—1·83
		Calcium balance			
1	5·51	4·54	3·13	7·67	—2·16
2	6·96	5·40	2·60	8·00	—1·04
3	6·73	5·71	2·70	8·41	—1·68
4	6·66	5·44	2·31	7·75	—1·09
Average	6·46	5·27	2·68	7·95	—1·49
		Phosphorus balance			
1	1·50	2·24	0·03	2·27	—0·77
2	1·76	2·36	0·03	4·39	—0·63
3	1·71	2·60	0·02	2·62	—0·91
4	1 70	2·43	0·03	2·46	—0·76
Average	1·67	2·40	0·03	2·43	—0·76

EXPERIMENT II

In view of the sub-optimal consumption of dry matter and pronounced negative balance of nitrogen, calcium and phosphorus under the dietary regimen of Experiment I, it was decided to repeat the trial, by introducing a supplement of half-a-pound of linseed-cake in the ration. The ration consisted of ½ lb. linseed-cake, 3 lb. maize-husk mixed with 1 oz. of common salt and with wheat-bhoosa fed *ad lib.* Each item was fed separately and any residue left of maize-husk and wheat-bhoosa was accurately weighed and recorded. As before, the ration was fed for a preparatory period of 15 days followed by a 10-day collection period. The animals were weighed as soon after as possible and at the end of the trial they recorded an average increase of 17 lb. each in live weight.

Food consumption. The consumption on dry basis of individual items of the ration is shown in Table VI. The data in Table VI show interesting features. The introduction of 201·2 gm. of linseed cake in the ration increased the dry matter consumption from an average 2020·4 gm. (Table II) to 2723·5 gm. This increase of about 35 per cent in dry matter consumption has been achieved almost entirely as a result of a larger intake of wheat-bhoosa. Consequent on this increased consumption

the intake per 100 lb. live weight has worked out at an average of 881 gm., a figure which is quite close to the expected normal.

TABLE VI

Food consumption per day (on dry basis). Experiment II

	Wheat bhoosa (gm.)	Linseed cake (gm.)	Maize husk (gm.)	Total consumption (gm.)	Consumption per 100 lb. live weight (gm.)
Hill Bullock No. 1	1124·0	201·1	1136·7	2461·8	820·6
Hill Bullock No. 2	1545·5	201·1	1185·9	2932·5	928·0
Hill Bullock No. 3	1425·1	201·1	1185·9	2812·1	889·9
Hill Bullock No. 4	1344·7	201·1	1141·5	2687·3	884·0
Average	1359·8	201·1	1162·5	2723·5	880·6

Results of metabolism. The chemical composition of feeding stuffs used, and the consumption, excretion and digestibility coefficient of various nutrients are detailed in Tables VII and VIII respectively. In Table IX is shown the balance of nitrogen, calcium and phosphorus.

TABLE VII

Percentage composition of feeding stuffs (on dry basis). Experiment II

	Organic matter	Crude protein	Ether extract	Crude fibre	N-free extract	Total carbohydrates	Total ash	Ca	P
Wheat bhoosa	83·42	2·99	0·69	37·23	42·51	79·74	16·58	·399	·114
Linseed cake	90·58	30·04	6·11	10·02	44·41	54·43	9·42	·583	·801
Maize husk	97·67	8·25	1·54	16·10	71·78	87·88	2·33	·304	·105

TABLE VIII

Digestibility coefficient of whole ration and maize husk. Experiment II

	Crude protein gm.	Ether extract gm.	Crude fibre gm.	Nitrogen-free extract gm.	Total carbohydrates gm.
			Bullock No. 1		
Consumed from—					
Wheat-bhoosa	33·57	7·79	418·42	477·81	896·23
Linseed cake	60·60	12·29	20·16	89·34	109·50
Maize husk	93·75	17·46	182·94	815·82	998·76
Total	187·92	37·54	621·52	1382·97	2004·49
Voided in faeces	85·43	14·86	226·81	367·91	594·72
Total digested	102·49	24·68	394·71	1015·06	1409·77
Digestibility coefficient of whole ration	54·5	66·4	63·5	73·4	70·3

TABLE VIII—*contd.*

	Crude protein gm.	Ether extract gm.	Crude fibre gm.	Nitrogen-free extract gm.	Total carbohydrates gm.
Digested from—					
Wheat-bhoosa	..	2·80	255·24	253·25	508·49
Linseed cake	51·51	11·80	5·44	59·86	65·30
Maize husk	50·98	8·08	134·03	701·95	835·98
Digestibility coefficient of maize husk	54·4	46·3	73·3	86·1	82·8
Consumed from—			*Bullock No. 2*		
Wheat-bhoosa	46·15	10·71	575·33	657·00	1232·33
Linseed cake	60·60	12·29	20·16	89·34	109·50
Maize husk	97·82	18·22	190·90	851·28	1042·18
Total	204·57	41·22	786·39	1597·62	2384·01
Voided in faeces	100·89	15·20	288·78	547·66	836·44
Total digested	103·68	26·02	497·61	1049·96	1547·57
Digestibility coefficient of whole ration	50·7	63·1	63·3	65·7	65·0
Digested from—					
Wheat-bhoosa	..	3·86	350·97	348·21	699·18
Linseed cake	51·51	11·80	5·44	59·86	65·30
Maize husk	52·17	10·36	141·20	641·89	783·09
Digestibility coefficient of maize husk	53·3	56·9	74·0	75·4	75·1
Consumed from—			*Bullock No. 3*		
Wheat-bhoosa	42·57	9·88	530·50	606·79	1136·29
Linseed cake	60·60	12·29	20·16	89·34	109·50
Maize husk	97·82	18·22	190·90	851·28	1042·18
Total	200·99	40·39	741·56	1546·41	2287·97
Voided in faeces	93·61	14·77	264·56	462·72	757·28
Total digested	107·38	25·62	477·00	1053·69	1530·69
Digestibility coefficient of whole ration	53·4	63·4	64·3	68·2	66·9
Digested from—					
Wheat-bhoosa	..	3·56	323·62	321·08	644·70
Linseed cake	51·51	11·80	5·44	59·86	65·30
Maize husk	55·87	10·26	147·94	672·75	820·69
Digestibility coefficient of maize husk	57·1	56·3	77·5	79·0	78·8

TABLE VIII—*concld.*

	Crude protein gm.	Ether extract gm.	Crude fibre gm.	Nitrogen-free extract gm.	Total carbohydrates gm.
	Bullock No. 4				
Consumed from—					
Wheat-bhoosa	40·16	9·32	500·61	571·66	1072·27
Linseed cake	60·60	12·20	20·16	89·34	109·50
Maize husk	94·16	17·54	183·73	819·38	1003·11
Total	194·92	39·15	704·50	1480·38	2184·88
Voided in faeces	93·35	14·77	245·54	487·12	732·66
Total digested	101·57	24·38	458·96	993·26	1452·22
Digestibility coefficient of whole ration	52·1	62·3	65·1	67·1	66·5
Digested from—					
Wheat-bhoosa	..	3·36	305·37	303·00	608·37
Linseed cake	51·51	11·80	5·44	59·86	65·30
Maize husk	50·06	9·22	148·10	630·40	778·55
Digestibility coefficient of maize husk	53·2	52·6	80·6	77·0	77·7

TABLE IX

Nitrogen balance

Animal	Intake gm.	Out put		Total gm.	Balance
		Faeces gm.	Urine gm.		
	Nitrogen balance				
Bullock No. 1	30·07	13·67	12·04	25·71	+4·36
„ No. 2	32·73	16·14	12·40	28·54	+4·19
„ No. 3	32·16	14·98	12·56	27·54	+4·62
„ No. 4	31·20	14·94	11·36	26·30	+4·90
Average	31·54	14·93	12·09	27·02	+4·52
	Calcium balance				
Bullock No. 1	9·12	5·08	2·56	7·64	+1·48
„ No. 2	10·95	6·58	2·92	9·50	+1·45
„ No. 3	10·47	5·74	2·64	8·38	+2·09
„ No. 4	9·91	5·13	3·00	8·13	+1·78
Average	10·11	5·63	2·78	8·41	+1·70
	Phosphorus balance				
Bullock No. 1	4·09	3·24	0·07	3·31	+0·78
„ No. 2	4·63	3·84	0·07	3·91	+0·72
„ No. 3	4·48	3·47	0·07	3·54	+0·94
„ No. 4	4·35	3·46	0·07	3·53	+0·83
Average	4·39	3·50	0·07	3·57	+0·82

DISCUSSION ON RESULTS OF COMPARATIVE METABOLISM TRIALS IN EXPERIMENTS I AND II

Digestibility Co-efficient. The introduction of a small quantity of linseed-cake into the ration had a profound effect on dry matter consumption and the digestibility of the whole ration, as also of maize-husk determined by process of elimination. The average digestibility co-efficient data summarized in Table X, show that the inclusion of the cake has improved the digestibility of crude protein and ether extract of the whole ration; the carbohydrates moiety being relatively little influenced. The effect seems to be much pronounced in the case of maize-husk, all the constituents of which have shown significantly higher digestibility in experiment II.

TABLE X

Digestibility co-efficient of the whole ration and maize-husk in Experiments I and II

	Crude Protein		Ether extract		Crude fibre		N-free extract		Total carbohydrates	
	W. R.	M. H.	W. R.	M. H.	W. R.	M. H.	W. R.	M. H.	W. R.	M. H.
Experiment I . .	41	47	28	26	61	61	70	74	68	72
Experiment II . .	53	55	62	53	64	76	69	79	67	79

W. R.—Whole ration
M. H.—Maize-husk

While it is desirable in determining the digestibility co-efficient of a test feed to reduce the associative effect of supplementary feed or feeds to a minimum, the co-efficient obtained of maize-husk under a simpler dietary in experiment I cannot be accepted as representative in view of physiological disturbances produced in the animals as evident from sub-optimal consumption and negative balances recorded of the important nutrients.

Balances of nitrogen, calcium and phosphorus. The data on balances of N, Ca and P have been summarized in Table XI. The supplementation of linseed-cake was responsible for the larger intake of nutrients not only from its own quota, but also from wheat-bhoosa, the consumption of which was stimulated by the inclusion of the cake in experiment II. The larger intake has a Salubrious effect on the balances (Table XI).

In experiment I, nitrogen utilization was about 22 per cent negative as more nitrogen was excreted in urine (10·17 gm.) than was absorbed (8·34 gm.), whereas in experiment II the utilization was positive to the extent of 27 per cent. This result is in conformity with the finding of Morris and Ray [1939] who showed no increased output of urinary nitrogen under conditions of phosphorus deficiency as in experiment I.

According to Sen, Ray and Talapatra [1942], the requirement of calcium of adult animals of 300 lb. body weight, fed on wheat-bhoosa and rape-cake, is easily met at a level of intake which is in the neighbourhood of 6 gm. per day. Although the average intake of calcium in experiment I was 6·46 gm. the balance proved to be significantly negative. This failure in the utilization can be ascribed to an extremely low level of ingestion of phosphorus and consequent wide ratio of Ca/P.

The results of the balance experiments definitely indicate that the low phosphorus content in maize-husk is a disturbing factor in its practical and economic utilization. The husk can be profitably fed with a staple roughage like wheat-bhoosa if it is mixed with a small quantity of linseed cake in the proportion of about 5 : 1.

TABLE XI

Balances of N, Ca and P in Experiments I and II

	Intake	Out-go		Total	Balance
		Faeces	Urine		
			Nitrogen		
Experiment I	—20·17	11·83	10·17	22·00	—1·83
Experiment II	—31·54	14·93	12·09	27·02	+4·52
			Calcium		
Experiment I	—6·46	5·27	2·68	7·95	—1·49
Experiement II	—10·11	5·63	2·78	8·41	+1·70
			Phosphorus		
Experiment I	—1·67	2·40	0·03	2·43	—0·76
Experiment II	—4·39	3·50	0·07	3·57	+0·82

TABLE XII

Digestible nutrients per 100 lb. dry and raw material

	Digestible nutrients per 100 lb. dry material				Nutritive ratio	Digestible nutrients per 100 lb. raw material		
	Crude protein lb.	Carbo-hydrates lb.	Ether extract lb.	Total lb.		Digestible crude protein lb.	Starch equivalent lb.	Total digestible lb.
Maize-husk	4·54	68·94	0·81	75·30	15·6	4·09	59·1	67·9
Wheat bran	8·72	59·10	1·14	70·39	7·1	7·85	54·5	63·4
Rice	6·76	35·15	10·00	64·40	8·5	6·08	52·0	58·0

The nutritive value of maize-husk

In Table XII is shown the digestible nutrients per 100 lb. of dry and raw maize-husk. For comparison, figures have been listed of by-products of similar origin from other cereals, namely, wheat

and rice. The data show that in available protein, the husk is relatively poorer than either wheat or rice bran, but in energy moiety it is somewhat richer than either of the by-products.

SUMMARY

Maize-husk or maize bran, apart from its total carbohydrates, is inferior in chemical make-up to wheat or rice bran. Contrary to other brans it is also extremely poor in phosphorus which is as low as 0·09 per cent.

When maize-husk is fed in a simple combination with wheat-bhoosa, the total dry matter consumption becomes sub-optimal, digestibility is depressed and balances of N, Ca and P are negative. This nutritional defect is corrected if the ration is supplemented by linseed-cake to an extent approximately one fifth of the maize-husk intake.

Under balanced feeding the digestibility co-efficient of maize-husk works out to be : crude protein 55, ether extract 53 and total carbohydrates 79. Based on these co-efficients, the nutritive value has been calculated as 4·09 lb., 59·1 lb. and 67·9 lb. digestible crude protein, starch equivalent and total digestible nutrients respectively per 100 lb. of the material.

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UTILIZATION OF BY-PRODUCTS OF STARCH MANUFACTURE FROM MAIZE AS CATTLE FEEDS

II. The nutritive value of maize-oil cake

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(Received for publication on 25 November 1947)

In Part I the authors [1946] described how the germs are separated from the kernels in the manufacture of starch from maize. To prepare maize-oil cake the germs are collected on metal sieves, dried first in roller mills and then in rotary steam driers after which the oil is expelled by oil expellers; the solid material left after oil extraction is the cake.

In this paper are presented the results of investigations on the nutritive value of maize-oil cake.

Chemical composition of maize-oil cake.

In Table I are given data as to percentage composition on dry basis of important organic and inorganic constituents of the cake. For comparison, similar figures are given for other oil-cakes commonly used in this country. It is seen that in its organic constituents, maize-oil cake is comparable with cocoanut-cake; while its inorganic constituents are more like those of groundnut cake. The ether extract content of 14·97 per cent is rather high for an oilcake used for feeding livestock. However, in view of the fact that cotton seed with an ether extract of 20·6 per cent [Sen, 1946] is tolerated and extensively fed to milch stock in certain parts of the country [Patel, Patel and Dave, 1944], the oil in the maize cake is unlikely to have an untoward effect on nutrition. The crude protein content is similar to that of cocoanut cake but lower than of other cakes. Of the cakes compared maize cake has the highest value for nitrogen-free extract which is also reflected in the value for the total carbohydrates. The richness in this constituent is natural in a by-product from a cereal, like maize. It is, however, poor in calcium and phosphorus and is the lowest in the group except groundnut cake.

Table I

Percentage composition on dry basis of maize and other oil cakes

—	Crude protein	Ether extract	Crude fibre	N-free exract.	Total carbo-hydrates	Ash	Calcium	Phos-phorus
Maize cake . . .	23·67	14·97	9·88	48·03	57·91	3·45	0·23	0·52
Cocoanut cake (country mill pressed).	23·44	13·00	12·91	42·28	55·19	8·37	..	..
Cocoanut cake (expeller)	25·34	8·20	13·20	44·92	58·12	8·34	0·36	0·66
Linseed cake . . .	30·51	6·57	9·48	43·24	52·72	10·29	0·37	0·96
Rape cake . . .	36·37	13·41	7·70	33·19	40·89	9·33	0·84	1·20
Til cake . . .	46·30	9·91	4·92	27·35	32·77	11·02	..	..
Groundnut cake . .	51·75	8·22	7·39	26·94	34·33	5·70	0·20	0·56

The data in Table II giving the composition of maize-oil cake produced in the United States [Morrison, 1937], United Kingdom [Halnan and Garner, 1944] and India, show certain well marked differences in their composition. Thus, the ether extract of the American variety is much lower, than that of either the Indian or British products while in other organic constituents it resembles closely the Indian cake. The British product is relatively poor in crude protein, but its total carbohydrate is higher and is also characterized by low crude fibre. The calcium content of Indian cake is significantly higher than of the two foreign products. In phosphorus, however, the Indian and American products are closely alike and richer than the cake produced in the United Kingdom. The differences in the composition of these products may be due to the chemical make-up of the raw material and to variations in the process of production.

TABLE VIII—*contd.*

Balances of nitrogen calcium and phosphorus—contd.

Hill bull	Intake	Out put			Balance
		Faeces	Urine	Total	
		Calcium balance			
1	10·44	6·86	0·96	7·82	+2·62
2	12·37	8·53	0·84	9·37	+3·00
3	11·95	8·60	0·80	9·40	+2·55
4	11·98	7·53	0·90	8·43	+3·55
Average	11·68	7·88	0·87	8·75	+2·93
		Phosphorus balance			
1	4·79	3·84	0·05	3·89	+0·90
2	5·23	4·41	0·04	4·45	+0·78
3	5·13	3·91	0·05	3·96	+1·17
4	5·14	4·36	0·05	4·41	+0·73
Average	5·07	4·13	0·05	4·18	+0·89

SUMMARY

In its organic constituents maize-oil cake is similar to cocoanut oil cake, while in inorganic make-up it is poor and resembles groundnut-cake. The results of the digestibility trial show that the digestibility co-efficients of maize-cake are, crude protein 84, ether extract 79, crude fibre 47, nitrogen-free extract 63, and total carbohydrates 60. Compared to that of the commonly used cakes the digestible crude protein is rather low. Under the feeding conditions of the present experiment using maize-cake and wheat-bhoosa , nitrogen, calcium and phosphorus balances were significantly positive.

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A NEW METHOD OF ESTIMATION OF OXALIC ACID IN BIOLOGICAL MATERIALS AND THE OXALIC ACID CONTENT OF INDIAN FEEDING STUFFS

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(Received for publication on 24 November 1947)

In majority of the methods published on the estimation of oxalic acid in biological materials [Dakin, 1907, Arbenz, 1917, Nelson and Mottern, 1931, Pucher *et al*, 1934, and Kohman, 1939], the principle involved is to release the acid free from its combination of a salt by treating the sample with a mineral acid. The free acid is then extracted with ether and from the ethereal extract, oxalic acid is precipitated as its calcium salt and finally estimated by permanganate titration. Some of these ether-extraction methods published on or before 1931 have been found not sufficiently accurate. Incomplete extraction of oxalic acid and the lack of proper control in its precipitation from the ethereal extract are factors responsible for inaccuracy in estimation. In later methods, particularly in that of Pucher *et al* cited above, these limitations have been successfully overcome and accurate and reproducible values have been obtained. The accuracy in estimation, however, is achieved only after introducing laborious and complicated modifications in the technique which are unsatisfactory for routine work involving at one time a large number of analyses. The ether-extraction method, moreover, offers a difficulty of considerable magnitude under the Indian conditions. In summer months, the complete extraction of oxalic acid by ether proves to be an impossible operation. Not only large amount of ether is wasted, but due also to much reduced number of syphoning, the extraction period has to be prolonged indefinitely and even then, uniformly satisfactory extraction cannot be made. Majumdar and De [1938] have attempted to simplify the extraction method by excluding the use of ether. Because of its simplicity, the method was given careful and repeated trials in this laboratory. These trials had however, shown that with biological materials, such as, cattle feeds, fodders and faeces, the method was unworkable. Further, in connection with some metabolism studies reported elsewhere [Talapatra, Ray and Sen, 1947], it was found essential to know the relative proportions of 'soluble' and 'insoluble' forms of oxalicacid occurring in oxalate-rich feeding stuffs. In the published literature there is no reference in which an attempt has been made to partition the oxalic acid of a food or other biological sample.

In view of the limitations in existing methods, and as none has scope in carrying out the partition a new method with the necessary advantage has been evolved. A part of the work reported in this paper was published in an abridged form by Talapatra *et al* [1942]; in the present communication the full details are being given and additional information regarding oxalic acid content in Indian feeding stuffs, its variation with the progressive maturity of the plant and its distribution in leaf and stem are also being included.

General principle of the new method. Depending upon the nature of the biological sample, it is first boiled in distilled water or in 1 per cent solution of caustic soda for half hour. Sufficient concentrated sodium carbonate solution is then added to make the final strength approximately 4 per cent and the boiling is continued for another half hour. During these two cooking processes, the following changes are effected; (*a*) the cellular tissues are completely broken down, (*b*) fatty matters are saponified and (*c*) by a process of metathesis, all insoluble oxalate salts are converted into soluble sodium oxalate. The liquid extract is then filtered and residue washed with hot water till the wash water does not show any alkaline reaction. The combined filtrate and wash water is then concentrated to a small volume and allowed to cool. In the cold, with constant stirring, hydrochloric acid (1:1) is added drop by drop till the final acid concentration after the neutralization of the alkali becomes approximately one per cent. At this stage, a heavy precipitate of extraneous matter appears which is allowed to flocculate. The extract is then carefully filtered. In the clear lightly coloured filtrate, oxalic acid is precipitated as its calcium salt and is determined in the usual way.

Details of technique. The biological material, such as a feeding stuff, is first dried and then finely powdered in a milling machine. A quantity varying from 2 to 5 grams of the powdered sample is taken in a 600 c.c. tall-form pyrex beaker and 40 c.c. of distilled water added. The beaker with its content is then placed over a sand bath, the top of the beaker being covered by a suitable round bottom flask filled with cold water to act as a condenser. As soon as the content in the beaker start boiling, it is given a thorough shaking and the boiling is allowed to continue for another half hour. At the end of this period keeping the beaker in the sand bath, 10 c.c. of a 20 per cent solution of sodium carbonate is added. The content of the beaker is thoroughly stirred with a rubber capped glass rod which is then washed in the beaker. The water in the condensing flask is also changed with fresh cool water. The cooking is then continued for another half hour. During the interval, the contents in the beaker are occasionally shaken without removing the condenser flask at the top. When the cooking is over, the content is filtered hot by suction through a disc of cloth (65 threads to an inch) fitted to a 7 c.c. Buchner funnel. The residue on the cloth is washed with hot water until the wash water does not show any alkaline reaction. The filtrate is quantitatively transferred back to the original beaker and boiled down to 100 to 150 c.c. It is allowed to cool, and then enough hydrochloric acid (1:1) is added drop by drop and with constant stirring until the final acid concentration becomes approximately one per cent. At this stage a flocculent precipitate appears and the whole thing after vigorous stirring is transferred quantitatively into a 200 c.c. volumetric flask and the volume is made up to the mark. The precipitate in the flask is allowed to settle and the clear supernatant liquid is filtered off through a fluted filter paper. An aliquot of the filtrate is taken in a 400 c.c. beaker, diluted with water to 200 c.c. and then made just ammonical and reacidified with acetic acid. In the cold medium is added 10 c.c. of a 10 per cent solution of calcium chloride and the mixture is stirred well to induce the precipitate of calcium oxalate to appear. The precipitate is allowed to settle overnight. The clear supernatant liquid is carefully decanted off through a Whatman No. 42 filter paper leaving as much of the precipitate as possible undisturbed. The precipitate is then dissolved in hydrochloric acid (1:1) after first allowing a few c.c. of the acid to pass through the filter paper and collecting it in the beaker containing the precipitate. From the hydrochloric acid solution, oxalic acid is reprecipitated and, this time, from a hot solution. The second precipitation is necessary because, in the first, some colouring matter is liable to be dragged along with the calcium oxalate precipitate which in subsequent titration with standard permanganate is likely to introduce an error.

The final calcium oxalate precipitate is dissolved in dilute sulphuric acid and then titrated with 0·05 N potassium permanganate solution. One c.c. of 0·05 N permanganate solution is equivalent to 0·00225 gram anhydrous oxalic acid. All figures of oxalic acid shown in this paper are expressed in the anhydrous form.

Estimation of oxalic acid in cattle faeces. Since cattle faeces contain relatively large amount of silica, it is necessary that this should be removed before oxalic acid is precipitated by calcium chloride. Besides this additional step, the slight modification in the method required for the purpose may be described as follows: 5 grams of the dried and finely ground sample of faeces are first cooked in 40 c.c. of one per cent solution of caustic soda for half hour which is followed by another half hour cooking with the sodium carbonate solution. After the cooking is over, the extract is separated by filtration through a disc of 7 c.m. Whatman No. 1 filter paper fitted to a Buchner funnel. The extract is boiled down to about 50 c.c. and allowed to cool. In the cold extract, enough hydrochloric acid (1:1) is added to make the final acid concentration one per cent. The precipitate formed is filtered off. A suitable volume of the filtrate is taken in a graduated flask and just sufficient ammonia added to make it alkaline. The volume is made upto the mark with distilled water and the content of the flask is thoroughly shaken. The silicic acid precipitate is filtered off and in an aliquot of the filtrate, oxalic acid is determined.

Estimation of water soluble oxalates. The obvious use of water for the extraction purpose is precluded by the fact that free organic acids are liberated from the plant material if cooking is carried out with water alone. The liberated acids are liable to act on some of the salts of calcium and magnesium and the cation thus set free will combine with soluble oxalates to form insoluble salts. The

extraction is therefore carried out with an ammonical solution so as to prevent the formation of free acids.

The details of extraction consist of as follows: A suitable quantity, 2 to 5 grams, of finely ground sample is boiled with an ammonical solution (1:10) under reflux for one hour. At the end of the period, the reflux is removed and the boiling is continued for another 15 minutes. The content is cooled and diluted with distilled water to make the extract approximately 200 c.c. After a thorough shaking, the content is allowed to rest so that the solid may settle at the bottom. The supernatant liquid is then filtered through a quantitative filter paper to eliminate the possibility of fine crystals of insoluble oxalate passing into the filtrate. The rest of the procedure is the same as described for the total oxalate estimation.

Experimental trials

In establishing the method as described above, the fulfilment of the following conditions is essential:

(*a*) The conversion of insoluble oxalates into soluble oxalates by metathesis with sodium carbonate should be quantitative.

(*b*) The cooking processes should guarantee complete extraction of salts of oxalic acid from biological materials.

(*c*) The method should yield reproducible values and effect the desired recoveries of the added pure salts of oxalate.

The conversion of insoluble oxalate into soluble oxalate. The insoluble oxalates in biological sample occur mainly as calcium oxalate. In small quantities, oxalates of other alkaline earth metals and perhaps in some cases micro quantities of manganese oxalate may also occur. To test the completeness of metathesis, a pure salt of calcium oxalate monohydrate (C_2O_4Ca, H_2O) was prepared in the laboratory. Duplicate weighings of the salt were directly titrated with 0·1N. permanganate solution after dissolving in dilute sulphuric acid. Another set of four duplicate weighings of this salt were first boiled with 4 per cent solution of sodium carbonate for 5, 10, 20 and 30 minutes respectively. The precipitate of calcium carbonate formed in an excess of sodium carbonate solution according to the equation $C_2O_4Ca+Na_2CO_3=CaCO_3+Na_2C_2O_4$ was filtered off and washed. The mixture of the combined filtrate and wash water was then titrated in the usual way with the standard permanganate solution. The amounts of permanganate solution required in the cases where no metathesis was carried and those where the expedient was used are shown in Table I.

It is apparent from the data presented above, that the estimated value is slightly higher than the calculated value but well within the experimental error. It may also be noted that the complete metathesis is brought about within such a short period as 5 minutes inspite of the fact that the quantities of calcium oxalate taken are much in excess of what can be expected in a biological sample. Similar results were also obtained when oxalates of magnesium, barium and manganese were tested.

The extraction of oxalates in biological samples. To determine the minimum time required for complete extraction of oxalates, a sample of finely ground paddy straw was taken. Three to five grams of the sample were first cooked in water for half hour, followed by second cooking in sodium carbonate solution for 15, 30, 60 and 120 minutes according to the technique described. The values of oxalic acid estimated from the extracts obtained from the different periods of cooking in sodium carbonate solution are given in Table II.

The data in Table II, show that there is practically no difference in the value of oxalic acid estimated in the extracts obtained between 15 minutes and 120 minutes of cooking. The closely similar values, moreover, suggest that the complete extraction of oxalates is assured if the digestion with sodium carbonate is fixed for half hour only. Various other biological samples such as, hays, straws from other cereals and millets, cattle faeces, etc., were also tried and the results were uniformly satisfactory.

TABLE I

Metathesis of sodium carbonate and calcium oxalate monohydrate

Observations	Calcium oxalate taken (gm.)	Time of boiling with 4 per cent carbonate solution	C. C. of O. 1N. $KMnO_4$ required		Percentage difference between estimated and calculated value
			For estimation	Calculated	
1	0·2085	Blank ;	28·70	..	..
2	0·1740	Ditto	24·00	..	..
3	0·0760	5 min.	10·50	10·46	+0·4
4	0·1400	Ditto	19·35	19·30	+0·3
5	0·1011	10 min.	14·00	13·93	+0·5
6	0·0770	Ditto	10·60	10·60	+0·6
7	0·0774	20 min.	10·70	10·67	+0·3
8	0·1000	Ditto	13·80	13·78	+0·1
9	0·1215	30 min.	16·80	16·74	+0·4
10	0·0770	Ditto	10·65	10·60	+0·5

TABLE II

The effect of different cooking periods with sodium carbonate on oxalate extraction

Observations	Duration of cooking	Per cent oxalic acid estimated	Percentage deviation from the mean of all observations
1	15 min.	1·36	—0·7
2	„	1·37	*Nil*
3	30 min.	1·38	0·7
4	„	1·35	—1·5
5	60 min.	1·37	*Nil*
6	„	1·38	0·7
7	120 min.	1·35	—1·5
8	„	1·37	*Nil*

Replications and recoveries. The experiment was carried out on the following lines.

(*a*) Oxalic acid in a sample of paddy straw was estimated from ten independent weighings.

(*b*) Different known quantities of oxalic acid in the form of sodium oxalate were added to a series of the same aliquot of a paddy straw extract and the recoveries of the added oxalate were determined by the proposed method.

(*c*) To different weighings of exactly 1 gram of a sample of paddy straw, varying quantities of oxalic acid in the form of calcium oxalate mono-hydrate were added and the recoveries were determined.

The results of the experiments are given in Tables III, IV and V and a perusal of these would show that closely reproducible values and the desired recoveries of the added oxalate are obtained by the use of the proposed method.

Table III

Replicate estimation of oxalic acid in paddy straw

Sample No.	Per cent oxalic acid	Difference from the mean	Percentage deviation from the mean	Remarks
1	1·406	0·004	+0·3	(*a*) Standard deviation : 0·013
2	1·406	0·004	+0·3	
3	1·405	0·003	+0·2	
4	1·396	—0·006	—0·4	
5	1·425	0·023	+1·6	
6	1·406	0·004	+0·3	
7	1·396	—0·006	—0·4	
8	1·406	0·004	+0·3	
9	1·372	—0·030	—2·0	
10	1·405	0·003	+0·2	(*b*) Standard error : 0·004

Table IV

Recovery of added oxalic acid (as sodium oxalate) from a paddy straw extract

Observations	Oxalic acid present in the aliquot of paddy straw extract (mgm.)	Oxalic acid from added sodium oxalate (mgm.)	Total oxalic acid estimated (mgm.)	Oxalic acid recovered (mgm.)	Percentage recovery of added oxalic acid
1	14·85	8·91	23·73	8·88	100
2	14·85	17·55	32·08	17·23	98
3	14·85	26·73	42·19	27·34	102
4	14·85	35·64	50·85	36·00	101
5	14·85	44·55	60·30	45·45	102

Table V

Recovery of added oxalic acid (as calcium oxalate monohydrate) from paddy straw

Observations	Oxalic acid in the straw (mgm)	Oxalic acid from added calcium mono-hydrate (mgm.)	Total oxalic acid estimated (mgm.)	Oxalic acid recovered (mgm.)	Percentage recovery of added oxalic acid
1	16·89	11·71	28·75	11·86	101
2	16·89	6·34	23·20	6·31	100
3	16·89	10·18	27·23	10·34	102
4	16·89	9·88	27·00	10·11	102
5	16·89	5·86	22·60	5·71	97

Oxalates in Indian feeds and fodders

Since Fincke and Sherman [1935] have shown that the poor utilization of calcium in spinach is due to its high oxalic acid content, considerable attention has been paid to the determination of oxalic acid in vegetables, fruits and other dietary elements generally used for human consumption [Majumdar and De, 1938 ; Kohman, 1939 ; Rau and Murty, 1942]. It seems from the available analytical data that no survey has been carried out on the oxalic acid content in the feeds and fodders used for feeding live-stock. As farm animals are herbivorous and oxalic acid is known to occur in plant materials, the importance of this analytical study is obvious.

By the method already described, a large number of feeding stuffs have been examined for their oxalic acid content and the results are shown in Table VI.

Table VI

Oxalic acid in Indian feeding stuffs

Name of the feeding stuff	Percent oxalic acid on dry basis
A. *Green fodder*—	
1. Lucerne (*Medicago sativa*)	0·35
2. Berseem (*Trifolium alexandrinum*)	0·30
3. Jwar (*Andropagon sorghum*)	0·45
B. *Cultivated grasses*—	
1. Rhodes grass (*Chloris gyana*)	0·25
2. Johnson grass (*Sorghum halpense*)	0·23
3. Sudan grass (*Andropogon sorghum var sudanansis*)	0·44
4. Napier grass (*Penisetum purpureum*)	3·30
5. Guinea grass (*Panicum maximum*)	2·20
C. *Indigenous grasses*—	
1. Bhaujura grass (*Apluda mutica*)	0·27
2. Spear grass (*Andropogon contortus*)	0·25
3. Kikiyu grass (*Penisetum clandestinum*)	0·29
4. Dub grass (*Cynodon dactylon*)	0·44
5. Anjan grass (*Penisetum cenchroides*)	0·35
6. Wooly finger (*Paspalum dilatatum*)	2·81
D. *Tree-leaf fodders*—	
1. Oak (*Qurens alba*)	1·13
2. Ber (*Zizyphus jujuba*)	2·40
3. Pastawanah (*Grewia oppositifolia*)	5·00

TABLE VI—*contd.*

Oxalic acid in Indian feeding stuffs

Name of the feeding stuff	Percent oxalic acid on dry basis.
E. *Straws*—	
1. Paddy straw	1·66
2. Ditto	2·05
3. Ditto	2·45
4. Barley straw	0·30
5. Wheat straw	trace
6. Jwar straw	0·31
7. Oat straw	trace
8. Regi straw	trace
F. *Grains, cakes and brans*—	
1. Maize	trace
2. Barley	0·10
3. Mustard cake	0·15
4. Rape cake	trace
5. Groundnut cake	0·19
6. Wheat bran	trace

It is evident from the data in Table VI that oxalic acid is present in Indian feeding stuffs from negligible to fairly high quantities. Some of the fodders, such as, paddy straw, napier grass and guinea grass, which are used extensively for cattle feeding are exceptionally rich in oxalic acid. In view of their high oxalate content, further examination was made of these foodders to study the distribution of ' soluble ' and ' insoluble ' oxalic acid and the minerals with which they may naturally occur in combination. The results of this examination are shown in Table VII.

TABLE VII

Oxalic acid partition and mineral content in some oxalate-rich fodders

Name of the sample	Total oxalic acid	Percentage composition on dry basis					
		Soluble oxalic acid	Insoluble oxalic acid	Ca	Mg	K	Na
Paddy straw (1)	1·46	1·25	0·21	0·31	..	..	..
Do. (2)	1·60	1·35	0·25	0·28	0·22	2·50	0·07
Napier grass (early cut)	3·05	2·04	1·01	0·81	0·51	2·90	0·11
Napier grass (dead ripe)	0·65	0·40	0·25	0·17	0·20	1·83	0·07
Guinea grass (early cut)	2·00	1·10	0·90	0·66	0·48	2·66	0·17
Guinea grass (dead ripe)	0·80	0·25	0·35	0·44	0·35	1·16	0·10

From the data in Table VII, it may be seen that (*a*) the major portion of the total oxalic acid is present in the soluble form ; (*b*) oxalic acid in these plant materials can combine with two to three times the calcium present in the plants to form insoluble calcium oxalate and (*c*) the distribution of the bases suggests that soluble oxalates are mainly in the form of potassium oxalate and the insoluble oxalates can be accounted for as calcium or magnesium oxalate or as a mixture of both. From the

metabolism experiments reported by Talapatra, Ray and Sen [1947], it would appear that the insoluble oxalates in paddy straw mainly occur as calcium salt.

The effect of progressive maturity of fodders on oxalate content. It may be seen from the data in Table VII that early cut napier grass contains more oxalic acid than one cut at the ripe stage. Same is the case with guinea grass. Since these cultivated fodder plants are fed both at young and mature stages, a systematic study was carried out to determine their content at progressive stages of maturity. On the basis of theoretical interest, similar study with paddy plant was also included. Besides oxalic acid, nitrogen and some of the major minerals were analysed in the sample. The results of this study are given in Table VIII.

TABLE VIII

The effect of progressive maturity on oxalic acid and mineral contents in some oxalate-rich fodders

Name of the fodder	Stage of cutting	Percentage composition on dry basis						
		Oxalic acid	Ca	P	Mg	K	Na	N
Paddy	Early	2·50	0·38	0·46	0·19	3·39	0·11	1·33
	Prime	1·92	0·32	0·48	0·19	3·00	0·07	1·40
	Flowering	1·78	0·33	0·50	0·23	..	0·08	0·94
	Milk	1·32	0·32	0·44	0·22	1·94	0·06	0·90
	1st ripe	1·10	0·30	0·45	0·21	1·66	0·07	0·82
	Dead ripe	0·90	0·27	0·28	0·18	1·49	..	0·35
Napier grass	Prime	3·30	0·43	0·79	0·50	4·50	0·15	1·70
	Flowering	1·60	0·23	0·47	0·32	2·70	0·11	0·60
	Ripe	0·68	0·17	0·35	0·23	1·88	0·07	0·37
	Dead ripe	0·65	0·16	0·32	0·20	1·66	0·06	0·28
Guinea grass	Prime	2·00	0·66	0·66	..	2·66	..	1·55
	Flowering	1·10	0·43	0·29	..	1·91	..	0·63
	Ripe	0·80	0·44	0·20	..	1·16	..	0·36

The data in Table VIII show that with the progressive maturity of paddy plant, the oxalic acid content steadily decreases. This decrease appears to be correlated with a similar fall in nitrogen content. If the figures for nitrogen is considered as the index of vegetative development, it can reasonably be assumed that the elaboration of the salts of oxalic acid is associated with the vegetative growth of the plant. Amongst the minerals, potassium alone seems to undergo similar change. Unlike that of paddy, the data on the analysis of napier and guinea grass show, that, with the progress from prime to efflorescent stage, the reduction of oxalic acid, nitrogen and potassium is relatively much more pronounced.

In view of the fact that animals while eating often show preference for the leafy portion of a fodder, a study was made of the distribution of oxalic acid, nitrogen and minerals in leaf and stem of napier grass cut at different stages of maturity. The results are set out in Table IX.

TABLE IX

Distribution of oxalic acid and minerals in stem and leaf of napier grass

Height of plant	Parts of plant	Percentage composition on dry basis					
		Oxalic acid	Ca	P	Mg	K	N
2 ft.	Leaf	3·28	0·56	0·61	0·39	3·10	2·58
	Stem	3·62	0·36	0·97	0·48	5·81	1·76
	Whole	3·50	0·43	0·79	0·50	4·50	1·70
5 ft.	Leaf	2·21	0·39	0·24	0·32	2·28	1·12
	Stem	1·46	0·11	0·54	0·29	2·86	0·45
	Whole	1·60	0·23	0·47	0·32	2·70	0·60
12 ft.	Leaf	1·54	0·34	0·23	0·18	2·06	1·00
	Stem	0·46	0·11	0·47	0·20	2·32	0·43
	Whole	0·68	0·17	0·35	0·23	1·88	0·37

The data in Table IX show that at the early stage, oxalic acid is slightly higher in the stem than in the leaf. But as the plant grows, the distribution is reversed and the difference in oxalic acid composition between the stem and the leaf becomes fairly wide. Nitrogen, as well as, calcium are higher in the leaf, whereas phosphorus is higher in the stem at all stages of growth. High phosphorus value in the stem appears to be a peculiarity of napier plant.

SUMMARY

1. In view of the limitations in the applicability of the existing methods of estimation of oxalates in biological materials, a new method has been described which is simple, quick and accurate and also permits, with minor modification, the partitioning of total oxalates into soluble and insoluble forms. The method mainly consists in digesting the sample with a strong alkali carbonate solution which not only breaks the tissue cells, thereby releasing the encased salts, but simultaneously converts by a process of metathesis, all insoluble oxalates into soluble salts. The total oxalate is thus brought into the extract. The extract is then separated by filtration and treated in the cold with hydrochloric acid to allow precipitation of the extraneous matters dissolved in the alkaline extract. The acid treated extract is filtered. In the filtrate, oxalate is precipitated by calcium chloride and finally estimated by the usual permanganate titration.

2. An analytical survey of the total oxalic acid content in commonly fed Indian feeds and fodders shows that it is present from negligible to a very significant quantity in different feeding stuffs. Some fodders, such as, paddy straw, napier and guinea grass are rich in this substance. In oxalate-rich fodders, the salt is present both in soluble and insoluble forms. The soluble form, which occurs mainly as potassium oxalate, represents by far the major portion of the total oxalate. The total oxalic acid content in these fodders decreases with the progressive maturity of the plant. In napier grass, at the early stages of its growth, the oxalate content in the stem is higher than in the leaf. With the maturity, however, this distribution is reversed.

The work described in this paper was carried out in the Animal Nutrition Section, Indian Veterinary Research Institute, Izatnagar.

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ABSTRACTS

I. Shope, R. E., Griffiths, H. T., and Jenkins, D. L. (1946). **Rinderpest. 1. The cultivation of rinderpest virus in the developing hen's egg.** *Amer. J. Vet. Res.* **7**, 133-134

II. Jenkins, D. L. and Shope, R. E. (1946). Rinderpest. VII. **The attenuation of rinderpest virus for cattle by cultivation in embryonating eggs.** *Ibid.* 174-178

III. Baker, J. A. and Greig, A. S. (1946). Rinderpest. XII. **The successful use of young chicks to measure the concentration of rinderpest virus propagated in eggs.** *Ibid.* 196-198

IV. Cooper, H. K. (1946). Rinderpest. XVI. **Complement fixation test for rinderpest.** *Ibid.* 228-237

I.

FERTILE eggs were incubated at a temperature between 37°-39°C and ten-day embryonated eggs were inoculated with 0·2 c.c. of a 10 per cent suspension of rinderpest calf spleen tissue in broth, the inoculum being dropped through a window cut in the egg shell over the region of the embryo. The window was sealed and the eggs incubated for three days and then opened for serial passages. The chorio-allantoic membrane and the amniotic membrane were removed aseptically and ground well with about 3 c.c. of the amnio-allantoic fluid; of this 0·2 c.c. was used for each passage. In this manner eleven serial passages were made. It was found that the virus was present in the membranes but not in the fluids and embryos.

II.

SERIAL passages were made by yolk sac inoculation as well as over the chorio-allantoic membrane. The authors observed that attenuation was more regular and uniform in virus passaged by yolk sac inoculation than in virus passaged by membrane inoculation. From the 67th onwards to the 71st passage the virus was found to be attenuated to a degree such that it could be safely used as a vaccinating agent, inoculated animals showing only a thermal reaction. Immunity was effective within 4 to 10 days. Vaccinated animals did not transmit infection to in-contact healthy animals.

III.

ANTISERA from chicks were obtained by inoculating intravenously the avianized rinderpest virus when the chicks were one day old and by bleeding the chicks 21 days later. The sera were tested in rabbits for neutralizing capacity against rabbit-adapted virus, the rabbits showing no thermal reaction if the virus was neutralized. Neutralization tests were successfully conducted and the chicks were good substitutes for calves when it was desired to determine either the presence of the avianized form of rinderpest virus or its concentration.

IV.

AMNIO-ALLANTOIC fluid collected from an infected egg constituted the antigen. Inactivations was achieved by heating the antigen at 60°C for one hour. By the use of this test the authors demonstrated complement-fixing antibodies in the sera of vaccinated animals from 14 days to 5 or 6 months after vaccination. (C.S.)

Max Kleiber (1945). Dietary Deficiencies and Energy Metabolism. *Nut.* Abstracts and Reviews 15(2), 207-222

MOST dietary deficiencies affect the efficiency of energy utilization. This effect is a measurable quantity and hence a better index of the economic importance of a deficiency rather than an ill-defined pathological condition in which a deficiency finally manifests itself..

The net utilization transfer of food energy to a form of energy useful to man—of all well-balanced rations is a maximum constant. As a deficiency unbalances a ration, the efficiency of energy utilization is decreased either through a decrease in food intake or an increase in the metabolic rate or the calorigenic effect of the diet.

Most mineral deficiencies decrease the appetite and increase the wastage of food energy as animal heat. Potassium, magnesium and calcium deficient rats require more food than the corresponding controls to attain the same body-weight. Same is true of cows maintained on a low phosphorous ration.

Vitamin deficient rats cease to grow before showing a decrease in their appetite. The decreased utilization is due primarily to lack of an anabolic principle in the vitamin and secondly to a depraved appetite. Among other things, vitamin C raises the power of endurance of the consumer. Vitamin B is specific in the production of fats from carbohydrates with an increase in oxidation and it also regulates the efficiency of muscular work. Riboflavin is related to the metabolic enzymes. Its deficiency in rats raised the calorigenic action of food and decreases protein and fat storage. But for slight lowering of the protein storage vitamin A deficiency does not affect the energy utilization. Recently, however, it has been observed that the higher protein storage in vitamin A supplied controls is accompanied by a higher retention of fat and water.

The catabolism and hence the partial calorigenic action of a particular aminoacid is decreased by its deficiency and this is true for all proteins. But in a protein-deficient-diet, the decreased catabolism is over-compensated by an increased oxidation of carbohydrates and fats. Expressed per unit of metabolic body size there is no difference in the metabolic rates of a deficient or a control rat. This rate of a deficient rat is observed to decrease or to increase according as the comparison is made against a control on an unlimited food-intake or against one whose food-intake is restricted to that of the deficient rat. The differences introduced by the body size and the reduced food intake are eliminated in the latter and is the *per se* effect of the deficiency. Even this *per se* effect is the sum total of several inter-related tendencies.

The potassium deficiency *per se* increases the metabolic rate. But for brain tissue the rate of oxygen consumption of the tissues *in vitro* is depressed by increasing potassium concentration in the medium.

Magnesium and calcium activate phosphatases. The latter is particularly responsible for liberating energy for muscular work by the breakdown of adenosine triphosphatase. The changes in metabolic rate on account of the deficiency of these minerals are only apparent. The *per se* effect in both the deficiencies being towards an increase. In general their increasing concentration depresses nerve respiration *in vitro*. The decreased calcium concentration stimulates the oxidation-rate of tissues *in virto*.

Lack of iron through anæmia impairs the oxygen-supply in the organism. However, as is evident from the contrary references available, the deficiency of this element has no significant effect on the metabolic rate.

Iodine as a component of thyroxine has an unique role in controlling the metabolic rate. Though iodine deficiency in rats tends to lower the metabolic rate, it is ingestion of the iodized salt that lowers the high metabolic rate during pregnancy.

Phosphorous is important in biological energy transfer. However the deficiency of this mineral produces no significant difference in the rate of oxygen consumption. *In vitro*, kidney slices have a higher rate in phosphorous-free-medium but the nerve tissues consume more oxygen on addition of phosphate. Ascorbic acid has only a doubtful significance in animal respiration. The literature on the subject does not give a satisfactory picture of the influence of the deficiency on the metabolic rate of animals or that of tissues *in vitro*.

Vitamin B_1 is significant in biological breakdown of carbohydrates. The deficiency of this vitamin has a depressing effect on the respiratory exchange of birds, hens and rats. The low metabolic

rate in deficient beings can be raised by a provision of the vitamin in the diet. The *in vitro* parallelism between tissues from vitamin-deficient and cyanide-poisoned pigeons, indicates lack of respiratory enzymesin vitamin B deficiency. Addition of vitamin B. or yeast extract raised the oxidation-rate of beriberi tissues. Polyneuritis has but a doubtful effect on tissue-metabolism.

Riboflavin is involved in major normal oxidation processes. A lack of this constituent leads to a complex disturbance of coenzyme functions. Riboflavin deficiency *per se* has no marked effect on basal metabolism of rats. . The limited *in vitro* study tilts in favour of the riboflavin deficiency lowering the oxidation rate.

A diet, deficient in vitamin A or having an excess of it, does not significantly alter the metabolic rate. A reduced oxidation rate observed in *in vitro* studies with most of the tissues from the vitamin deficient rats points to the possible role of the vitamin in the oxidation of cells.

Administration of vitamin D raises the metabolic rate and in keeping with this, the muscle from rats administered liberally with this vitamin has a higher rate of oxygen consumption *in vitro*. The skin from rachitic rats has a low oxidation rate *in vitro*.

The major role of vitamin E has not yet been proved adequately. The vitamin is essential to the integrity of skeletal muscle and its deficiency leads to muscle dystrophy. The vitamin deficient rats exihibit a metabolic increase which can be lowered to the normal level by addition of wheat-germ oil or L-tocopherol to the deficient diet. Nevertheless, there is evidence to show that the potential metabolic increase in deficient rats is just counter-balanced by the opposite effects of a lowered food intake. *In vitro* studies on the dystrophic muscles of rabbits, guinea-pigs and rats support the view that the vitamin E deficiency increases the metabolic rate which is lowered by addition to the medium of L-tocopherol. A recent contradiction of this view needs elucidation.

On account of the universality of their functions, as enzymes, hormones and antibodies, the protein deficiency lacks well-defined symptoms. The metabolic effects of a protein or a single amino-acid deficiency are not fully known. The view, that protein deficiency *per se* lowers the metabolic rate is based on meagre grounds. No data are available on the *in vitro* metabolism of tissues from protein deficient animals. However, it has been observed that addition of protein in any form to most of the tissues of normal animals increases the oxidation rate.

It has been mentioned before that a low protien diet necessitates an increased oxidation of carbohydrates and fats. The loss of energy thus incurred is a homeostatic waste.

Metabolic stimulation in most mineral deficiencies *per se* may also be due to homestatis. As in a normal animal the mineral concentration in the soft tissues remains nearly constant, the deficiencies can apparently be made up by destroying the muscles and hence there is the observed increase in the oxidation rate.

Vitamin deficiencies in general do not affect or decrease the metabolic rate. Probably, the vitamins can considerably be squeezed out of the tissues without destroying them and so from an economic view point, the vitamin deficiencies need not be attended to as much of those of minerals.

(S.S.N.)

(I) MURNANE *D.* (1945). **A preliminary report on the treatment of clinical and sub-clinical streptococcal and staphylococcal infections of the bovine udder with penicillin.** *Aust. Vet. J.* **21,** 82

(II) MURANE *D.* (1946). **Second report on the treatment of clinical streptococcal and staphylococal infections of the bovine udder with penicillin.** *Ibid.* **22,** 35

(I)

PENICILLIN administered by udder infusion proved efficacious in treating 134 quarters of 60 cows in 10 commercial herds infected with streptococcal mastitis. The duration of treatment was six months. One single dose of 100 c.c. penicillin infusion in normal saline with a concentration of 150 units per c.c. when introduced into the udder, at low pressure just after milking, eliminated

infection from 37 per cent and two such doses from 72 per cent of clinical cases. Two further treatments in persistent infections cured 80 per cent and this repeated in the reminder gave a 93 per cent cure.

Nearly 95 per cent of the penicillin injected was absorbed from the udder tissue in about 12 hours but an effective concentration remained after this period if the dose was not less than 15,000 units.

Treatment resulted in a transient fall in milk yield 13-37 per cent. The bacterial, and cell counts were practically nil within 12-24 hours of treatment. Staphylococcal infection was less amenable to treatment.

Stress is laid on maintaining a high standard of shed hygiene and milking methods if the penicillin treatment is to prove effective.

(II)

Results are given of further trials with penicillin, treating six batches of 158 streptococcal and staphylococcal mastitis cases by udder infusion. A preliminary dose of 25,000 units of penicillin in 100 c.c. normal saline followed by two doses of 5,000 units at 12 hourly intervals cured 71 per cent of clinical streptococcal mastitis cases. The percentage cure with three doses of 25,000 units in distilled water was 78 to 93 in clinical mastitis due to *Str. agalactiae*, 100 due to non-group B, streptococci and nil due to staphylococci. One single dose of 100,000 units in distilled water eliminated infection in 38 per cent of clinical mastitis due to *Str. agalactiae*, in 25 per cent due to streptococcus non-group B and in none due to staphylococcus. Three doses of 12,000 units in distilled water at 24 hour intervals eliminated infection in 71 per cent of clinical mastitis due to *Str. agalactiae* and in 17 per cent due to non-group B. streptococcus. Staphylococcal mastitis did not respond to treatment. Four doses of 25,000 units in distilled water were tried in treatment of 2 cases of clinical staphylococcal mastitis, without success. Three doses of 25,000 units in sterile peanut oil at 24 hour intervals proved efficacious in eliminating infection from 94 to 100 per cent cases of clinical mastitis due to *Str. agalactiae* but staphylococcal mastitis was not suppressed.

It was observed that an effective concentration of penicillin within the udder for two-three days was more beneficial than a higher concentration for a shorter period. Although more than three doses enhanced the percentage cure, it was not economical for the farmer.

Emphasis is laid on the inadequacy of penicillin alone in reducing the incidence of mastitis in commercial dairy herds, other factors, particularly hygienic, are of dominant importance. (M.M.S.)

ROBERT L. SQUIBB (1946). **A New Method for the Control of Cattle Ticks in Tropical Regions.** *J. Am. Sci.* Vol. 5, No. 1, February 1946, 71-79

THIS paper reports the efficiency of a new combined solution of D.D.T. and derris for use as spray in the control and eradication of cattle tick in tropical areas. Two solutions A and B are prepared separately from D.D.T. and derris and used as a combined spray. The two solutions are prepared as follows.

D.D.T.	6·16 gms.	Green crushed derris roots . . .	22·5 gms.
Kerosine	66·6 c.c.	Water	200 c.c.
Water	33·3 c.c.	Neutral soap	3·0 gms.
Neutral soap . . .	1·8 gms.		

(*a*) Dissolve D.D.T. in kerosine and warm the solution. The water and neutral soap are mixed and heated to 98°C. This is then well mixed with D.D.T. solution. (*b*) Green crushed derris roots or derris powder containing 5 per cent rotenone solution is extracted in water for 24 hours and filtered. The neutral soap is dissolved in hot water, cooled and then added to the rotenone solution. Solution B should be prepared just prior to use. The spray is made by mixing one part solution A to one part solution B.

From the results of several tests this solution was found to have excellent possibilities for use as a spray in the control of cattle ticks. It was not poisonous and did not produce any irritation. For general eradication of ticks in an infested area each animal may be sprayed with 80 to 150 c.c. per head of the combined solution every 14 days. It effected a 85 to 99 per cent mortality of ticks within 7 days after the application of the spray. If desired, the combined solution may be used with the same effect diluted with equal quantity of water. The solution could be applied on animals by use of hand or knap sack sprayers and power sprayers. It is economic and easier to apply and acts more rapidly in complete eradication of ticks than the control methods hitherto used in tropics. It averts danger of losses due to overheating, mechanical injuries and poisoning.

The D.D.T. rotenone combination is superior to the use of either ingredient alone. It works well under both dry and wet tropical conditions and even a continuous rainfall of eight hours following the applications of the spray did not reduce its efficacy very much. A fine penetrating spray was found most efficient and the results depended very much on the spray technique. The progressive killing action of the combined solution lasted as long as seven days. (P.B.M.)

MGIPC—M—III-1-4—5-11-48—520

RESEARCH INTO CONTAGIOUS ABORTION OF CATTLE, SHEEP, AND GOATS*

BRUCELLOSIS IN INDIA

By JOHN B. POLDING

(Received for publication on 6 June 1947)

INTRODUCTION

THE scheme for the investigation of contagious abortion in the cattle, sheep and goats of India has occupied a period of five years, involved a considerable monetary expenditure and entailed some 25 thousand miles of travel, in pursuing an enquiry throughout a subcontinent. Consequently, to avoid costly repetition and to preserve hardly won information, it seems that the results should be recorded as a single consecutive entity. In compiling this report, therefore, an attempt has been made to give a comprehensive, but concise, exposition of all that has been learnt during the scheme. Further, although some of the work has been partially or wholly described elsewhere—in annual reports or articles—nothing has been omitted on that account, which would detract from the continuity or completeness of the narrative. Finally, for the convenience of future workers, a record of standard laboratory methods, experimental details and standing technical instructions has been interpolated in small print.

The scheme has not primarily been one of pure research, but was chiefly intended to bring the problems of Brucellosis in India into line with similar problems overseas; the work has, therefore, been mainly a collecting of material and a collation of information, which resulted in examinations, classifications, and comparisons, in their turn inevitably leading to conjecture. It is regrettable that owing to a paucity of funds for pure research and a shortness of time, conjecture could not always be tested by planned experiment; nevertheless, where suppositions seem usefully to point the way, they have not been excluded from this paper.

The period of the scheme was utilized thus. The first year was devoted to the collection of apparatus, the training of staff, the examining of old records and to general preliminary organization. The next three years were almost entirely occupied by survey work, although towards their end elementary vaccination was being introduced. Into the last short year (May 31st, 1943 to March 31st, 1944), was crowded most of the purely experimental work which arose out of the earlier experience and, with so much to be done in so short a time, much of this endeavour has suffered from haste and incompleteness.

To render a report dealing with a peculiar environment concise enough to please the local reader and at the same time to introduce enough local colour to make the matter intelligible to overseas workers, is always an inenviable task; it is regretted, if the geographical and climatological details of the resulting conspectus seem superfluous to the one, or insufficient to the other.

SECTION I. EPIDEMIOLOGY, THE OCCURRENCE OF BRUCELLOSIS IN INDIA

Mode of occurrence

BRUCELLOSIS in India, as in other countries, may take the form of (1) a primary epidemic (2) an endemic and (3) an exacerbation of an endemic, amounting for a short period, to a secondary epidemic. Primary epidemics, though fortunately comparatively rare, can be of considerable severity and may produce an abortion rate of from 10 to 20 per cent of breeding stock in the course of a few months; such mishaps, moreover, are usually followed by not easily eradicable endemics. Conversely, endemics are exceedingly common in many Indian farms and villages and they usually take the form of a small but unending trickle of abortions at a rate of 1 to 5 per cent loss of calves

* Final report of the Imperial Council of Agricultural Research Scheme on Contagious Abortion, 1939-1944.

per annum. In special circumstances, however, major endemics are encountered and here the annual loss of calves may amount to 20 per cent of breeding stock during a period of several years; happily, such occurrences are again comparatively rare. Superimposed, or secondary, epidemics are not uncommon and in these, the loss of calves may amount to 5 to 15 per cent of breeding stock in the course of a few months.

Continental survey, a general conspectus

Fig. 12 gives an approximate representation of the geographical distribution of Brucellosis in India.

Most of the organized farms of the country have been visited while from most of the remainder specimens have been sent to Mukteswar for examination and it has been found that, with very few exceptions, Indian farms are to a greater or lesser extent infected with Brucellosis. Further, as the majority of strains isolated from farms have proved to be the pure European type (*Br. abortus*), the source of a good part of the infection is attributable to the earlier stock, much of which originated in Europe. The actual appearance of Brucellosis in these farms, therefore, is of small epidemiological significance, for, its occurrence is largely fortuitous depending on where the farms happen to be and the character of their foundation stock.

In the villages, on the other hand, the position is entirely different. Obviously, it has only been possible to visit a representative few rural areas in each province, but, nonetheless, discoveries in villages have been of far greater epidemiological interest than those in farms. If it is to be believed, for instance, that villages have become infected from farms, it would be reasonable to assume that most village infection would be found adjacent to farms. A glance at Fig. 12 shows that such is not the case, and indeed, village infection is commonest where farms are fewest and *vice versa*. Moreover, as is shown on page 47 all *Brucella* strains, so far isolated from villages, differ in their typing characteristics from the *Br. abortus* strains isolated from farms. It would appear, therefore, that village infection, as seen almost exclusively on the Indian peninsula, has not been imported from Europe *via* the farms, but is an indigenous Indian infection, the distribution of which is of the highest epidemiological significance. Be that as it may, the distribution and persistence of a bacterial disease is chiefly concerned with microbial dissemination and, not until the section dealing with this subject is reached, can a hypothesis covering the peculiarities of epidemiology of the disease in village be preferred.

In the meantime, a few remarks are necessary on some possible inaccuracies of village survey work. In a country of the utmost conservatism, where there are no laws giving power over livestock, and where religion frequently forbids the drawing of bovine blood, to make a serological diagnosis on privately owned animals is often impossible. It follows that village blood samples can only be obtained when the local veterinary staff are able to over-persuade the prejudices of the people. Furthermore, persuasion usually results in sick, rather than healthy, animals being produced and consequently cattle with some history of disease (in this case usually genital) are the ones most frequently bled. When, therefore, it is stated in the succeeding pages that say 45 per cent of animals tested were *Brucella* positive, this is not ordinarily a true percentage of all the stock of the village, but only of those more likely to be infected. Nevertheless, inaccurate though they may be, such records are the best obtainable and have been found by experience to reflect fairly exactly the apparent abortion rate in the villages concerned. As to the real abortion rate, in the absence of records, it is possible only to guess but where *Brucella* reactors are say upwards of 30 per cent, it is believed that abortion may occur at the rate of about 10 per cent per annum. The obtaining of blood samples from slaughter-houses is also often opposed, the chief trouble here being the disinclination of butchers to be put to the slightest inconvenience. However, the returns from tests of such samples are possibly only a very rough index of the prevalence of abortion in the surrounding villages and so the loss of samples of this sort is annoying but hardly calamitous.

Now it must be admitted at once that by far the largest number of village blood tests have been made in Madras, Orissa and the Central Provinces and Berar, or in other words in the very situations where village Brucellosis has been most diagnozed (Fig. 12), whilst in the United Provinces,

Punjab and North West Frontier Province tests have been disappointingly few. It is carefully to be considered, therefore, whether the results shown in the figure are, on this account, invalid. Leaving aside for the moment the serological test, reports of actual clinical abortions in villages are undoubtedly received voluntarily in the south-east, whilst in the north-west it is impossible to extract an admission from proprietors that such an occurrence is other than very rare. Now the villagers in the south-eastern tracts are often very orthodox and sometimes shy and ignorant too and it seems unlikely that they would describe an occurrence unless it actually happened. Moreover, village headmen will more or less willingly produce some 15 to 20 animals alleged to have aborted. So that the common finding of a high proportion of strongly positive blood tests amongst these 'aborters', together with the occasional isolation of a distinctive *Brucella* variant, surely corroborates the villager's statements.

In the north-west, however, the inhabitants are often well-informed and capable zemindars, yet they deny that abortion is common, resist blood sampling and in other words cannot be bothered with a subject the importance of which has evidently not been made plain to them by actual losses. Such tests as can be made hereabouts reveal only occasional doubtful, or more rarely, weakly positive reactors.

The inference is that the incidence of village infection as shown in Fig. 12 is, as far as it goes, correct; nevertheless, whilst it can be generally accepted that village infection is rare in the north-west and common in the south-east, it is of course highly probable that more centres of village infection remain to be discovered, especially in the extreme east, centre and south-west. Lastly it must be always borne in mind that it is not suggested that Brucellosis is totally absent from village cattle in the north-west; it is only stated that the incidence appears to be insignificant.

*A detailed survey with reference to climatic * regions*

A detailed description of the occurrence of Brucellosis, province by province, would be of scant general interest. So, because it is believed that the disease occurs with a certain relation to climate, a synopsis of its local incidence in regions of similar climate and character is given in Table I, while, for the interest of local workers a detailed place-to-place survey, together with notes on climate are interpolated in small print at the end of the sub-head.

TABLE I

Regional distribution of Brucellosis in India, with reference to climate

Region	Character of region	Annual rainfall	Brucellosis in	
			Farms	Villages
Baluchistan, North-West Frontier Province, Rajputana	Desert and arid hills .	Uni-seasonal, 5-20 in.	Minor epidemics or absent (farms infrequent)	Negligible
Punjab, United Provinces, Bihar	Cultivated plains .	Uni-seasonal, 15-40 in.	Moderate endemics (farms abundant)	Do.
Bengal, Assam . .	Deltaic cultivation and forest hills	Bi-seasonal, 50-100 in.	Moderate endemics (farms infrequent)	Not known
West Hyderabad, Berar and West Central Provinces	Cultivated plateau and forested hills	Uni-seasonal, 25-50 in.	Minor endemics (farms infrequent)	Negligible
East Central Provinces, East Hyderabad, Orissa, Madras, Mysore, South Bombay	Richly-cultivated coastal belts, with hilly interior	Tri-seasonal, 25-50 in.	Minor endemics or absent (farms infrequent)	Abundant
Central and North Bombay	Cultivated coastal belt, with hilly interior	Uni-seasonal, 25-100 in.	Major or minor endemics. Major epidemics (farms abundant)	Negligible

* The climatological data used in this section are taken from the Imperial Gazettier of India, Vol. XXVI, Oxford 1931, Clarendon Press.

A study of the detailed survey suggests that, as far as farms are concerned, Brucellosis is most prevalent where farms are abundant, but there is some evidence to suggest that, provided there is a sufficient reservoir of infection, epidemics and endemics in farms are gravest where rainfall is either very heavy, as in the coastal belt of Bombay and in Assam, or, to a lesser degree, in the bi-seasonal or tri-seasonal rainfall areas, such as the Himalayan water-shed, east of Meerut, Ambala and Lahore.

As for infection in village stock, inspection of Table I reveals a curious and interesting position. Infection, as far as it has been discovered, lies mainly in the tri-seasonal rainfall area of the peninsula south-east of the red line, Fig. 12. It has, however, advanced as far as, and follows with considerable accuracy the limits of the bi-seasonal rainfall area (south-east of the blue line, Fig. 12). Conversely, the area of minimum village infection seems to be the arid north-west. But, although, throughout these epidemiological studies, it is necessary to bear in mind the fundamental function of climate, its precise role can only be postulated when all other considerations have been brought into account.

A detailed survey of Brucellosis in India by climatic regions

Region 1. Sind, Baluchistan, North-West Frontier Province, Rajputana and Ajmer

This region consists of the Thar desert, the partially irrigated lower Indus valley and the arid hills of the north-west; the mean annual rainfall is the least in India.

Village stock. In this region, village *Brucellosis* seems to be rare or absent. Thus enquiries by the Veterinary Investigation Officers of Sind and the North-West Frontier Province and the writer elicited the invariable reply that village abortions occurred with extreme rarity. Again random tests at Peshawar, Karachi and in the Rajputana States showed a few doubtful or weakly positive reactors. Abortion in sheep and goats, on the other hand, is relatively common, but all tests on these animals have been negative to Brucellosis and the mishap can usually be attributed to other causes (page 189).

Farm stock. In the few organized farms of these parts, clinical abortions and *Brucella* reactors are either absent—as in the indigenous herds of Government or private farms; or present in a small degree—as in the cross-bred herds of the military farms scattered along the north-western foot-hills from Quetta to Peshawar. Apart from an occasional superimposed epidemic, the infection in these farms is endemic and the abortion rate is estimated to be less than 2 per cent per annum.

Region 2. The Punjab and the United Provinces

The major portion of this region is cultivated. The rainfall is moderate (40 in.) on the north-east, but decreases towards the west and south, where desert conditions tend to link up with region (1). In these southern and western tracts the rainfall is less than 2 in. with the exception of the period from June to October. Except on the Himalayan foot-hills, deep forest is rare.

Village stock. Apart from certain serious and anomalous epidemics in buffaloes, which are fully described on page 188 both clinical abortions and *Brucella* reactors are rare amongst village cattle. Enquiries in villages tend to put the rate of clinical abortion in cows at 1 per cent per annum or less, while random blood samples collected from slaughter-houses at Rohtak, Lahore, Meerut and Bareilly reveal a few doubtful and weakly positive reactors. For example, of 35 buffaloes bled at Rohtak none was positive; and of 292 buffaloes tested at Meerut, 10 (3·4 per cent) were doubtful reactors and three were weakly positive, whilst at Bareilly of 53 tests on cows and buffaloes two gave doubtful reactions. Goat and sheep abortion, however, is fairly common, especially in the semi-desert tracts of the west, but, here again, tests have always been *Brucella* negative and the condition is usually attributable to other causes.

Farm stock 1—Indigenous. In the indigenous herds of military, Government and private farms of the semi-desert tracts of the south-western Punjab, *Brucella* reactors are either present in small numbers (5-15 per cent of the herd), or were at one time present and have since died out. In the former farms, the abortion rate is about 1 per cent per annum and in the latter, although at one time it was as much as 7 per cent per annum, the rate is at present negligible. In the indigenous herds of the United Provinces, *Brucella* reactors are few (viz., about 5 per cent) and clinical abortions occur at the rate of less than 1 per cent a year; moreover, exacerbations have not been reported during

the last decade. Similar results have been obtained in the past by an examination of private gowshalas of this province.

Farm stock 2—Cross-bred. In the cross-bred herds of Government, military and private farms of this tract the abortion rate, with one or two exceptions, averages between 2½ per cent and 3 per cent per annum. Moreover, until the beginning of the wartime expansion at least, there is evidence to show that, co-incident with improvement in animal husbandry, the abortion rate was slowly decreasing. It is noticeable, however, that the endemic abortion rate is slightly higher in farms situated in, or close to, the Himalaya.

The incidence of *Brucella* reactors in 212 non-aborting cows taken at random from eight herds of this tract in which abortion is endemic, was approximately 20 per cent; in 118 aborting cows from the same herds, it was 51 per cent and, in view of the deficiencies of the *Brucella* test, it is safe to conclude that some 75 per cent, or more, of all abortions are due to Brucellosis. In this region, however, there are three exceptionl herds excluded from the above calculations. Here, exacerbations of Brucellosis have been occurring for the last few years and some rates are given in Tables II-IV.

TABLE II

Abortions at farm I

Year	Herd strength	No. of abortions	Percentage of abortions
1940	129	13	10·0
1941	134	16	11·9
1942	118	7	5·9
		Mean	=9·0

TABLE III

Abortions at farm II

Year	Herd strength	No. of abortions	Percentage of abortions
1940	137	17	12
1941	124	6	5
1942	96	19	19
		Mean	=12·0

About 50 per cent of the total stock of the above herd were infected with Brucellosis and in one test 82 per cent of aborting animals were found positive.

TABLE IV

Abortions at farm III

Year	Herd strength	No. of abortions	Percentage of abortions
1939	26	3	11·6
1940	30	3	9·6
1941	32	2	6·2
1942	36	4	11·1
		Mean	=9·6

It is clear from these results that endemic Brucellosis may flare up into an epidemic and that the consequent abortion rate, for a year or two at least, can be as high as 12 per cent of breeding stock.

Farm stock 3 Buffaloes.—Many of the organized farms just discussed carry herds of buffalo, as well as cows, and Brucellosis appears to be endemic in these animals also. In the eight mildly endemic farms mentioned in the last sub-head, the abortion rate in buffaloes worked out at about 1 per cent per year, whilst *Brucella* tests of 162 healthy buffaloes revealed five (3 per cent) reactors and of 36 aborting buffaloes eight (22 per cent) were positive. This remarkably low occurrence of *Brucella* reactors will be discussed on page 153.

As in cross-breds, so in buffaloes this region offers instances of severe epidemic abortion. Two occurred in farms I and II of the last sub-head, while the third is an entirely separate case. The records of these outbreaks are shown in Tables V-VII.

TABLE V

Abortions at farm I (buffaloes)

Year	Herd strength	Total abortions	Percentage of abortions
1940	178	3	1·7
1941	232	9	3·8
1942	307	12	3·0
		Mean =	2·8

It is to be noted that several *Brucella* strains were isolated from the buffaloes of this farm during the period 1940-42.

TABLE VI

Abortions at farm II (buffaloes)

Year	Herd strength	Total abortions	Percentage of abortions
1940	225	6	2·5
1941	327	10	3·0
1942	432	52	12·0
		Mean =	5·8

In this herd, about 48 per cent of the total buffalo stock was *Brucella* positive in 1940, whilst tests of 32 recently aborting buffaloes in 1942 revealed but 15 (48 per cent) positive. If, however, doubtful reactors were to be included this figure would rise to 70 per cent.

TABLE VII

Abortions at farm IV (buffaloes)

Year	Herd strength (average)	Total abortions	Percentage of abortions
1940			
1941	300	17	5·7
1942	300	9	3·0
	300	3	1·0
		Mean =	3·2

Farm stock & Goats.—There are two organized goat-farms in this region and in years gone by both were infected with Brucellosis. Sometime ago, however, one farm adopted a rigid half-yearly test-and-disposal scheme resulting in a partial eradication of infection and abortions have been avoided for several years. This herd is fortunate in that it is self-contained and has no co-habiting cattle, which might be a source of reinfection. In both these respects the second herd is not so well off and, although it is irrefutable that Brucellosis is endemic in these goats, it has been keenly disputed whether it is the cause of the abortions; so that, specificity being in doubt, the discussion of this farm is relegated to the non-specific section on page 189.

Region 3. Bengal and Assam

The south and south-west of this area is deltaic, water-logged and fertile, but to the north and north-east it is bounded by vast mountain ranges. From November to February the rainfall is negligible, from March to May it is moderate, while from June to September the rainfall, in parts, is amongst the heaviest in the world. Vegetation is everywhere plentiful and to the north and east forests abound.

Village cattle. As far as villages are concerned it must be at once conceded that this region has been very poorly surveyed, mainly because of bad communications and partly because of the absence of preparatory work by the local staff; later on account of the war. From enquiry, it seems that village abortion occurs from time to time and in the only two places where cattle of the village type were accessible (Shodepur gowshala and a village near Dacca) blood tests revealed positive reactors.

Farm cattle. There are not a great many organized farms in this tract and clinical abortion was not found amongst the indigenous farm stock of Bengal. However, although the true facts could not be ascertained, Brucellosis is probably present in the cross-bred cattle of at least one Calcutta dairy, whilst in past years a severe epidemic occurred in the European cattle of a dairy concern near Darjeeling. In Assam, on the other hand, four organized farms were all found to be infected with Brucellosis. Positive reactors occurred at the rate of 20 per cent of those tested and the abortion rate was between 2 per cent and 6 per cent per annum. It is significant that three of these farms carried herds of indigenous cattle and thus, for the first time, a noticeable abortion rate is encountered in well kept zebu cows. The stock of these farms is imported from northern India, but, in the past, European cross-breds have been in occupation.

Region 4. Berar, the Central Provinces, north and west of Akola and Hyderabad State, west of Warangal

The south-western part of this tract is situated on the Deccan plateau and the north-western parts are hilly and forested. Much of Berar and the adjacent parts are richly cultivated. The rainfall is less than 2 in. for three seasons of the year, but in the fourth season it is abundant.

Village stock. Clinical abortions in cattle, sheep and goats are rare in this tract and a few random blood examinations support this statement.

Farm stock. Most of the organized farms of this region carry indigenous stock and, in nearly all, the abortion rate is negligible; nevertheless, occasional *Brucella* reactors occur. There are two exceptions, one a cross-bred herd at Secunderabad, where the abortion rate is 2·8 per cent in cows and 0·1 per cent in buffaloes and the incidence of reactors is 8 per cent and 4 per cent respectively; the other is an indigenous herd in northern Central Provinces, where the abortion rate in buffaloes and cows alike was about 10 per cent during a *Brucella* epidemic occurring in 1940.

Region 5. The Central Provinces, east of Akola, Hyderabad State, east of Warangal, Orissa, Madras, Mysore and southern Bombay.

This region consists of a relatively hilly and forested hinterland, incized by valleys running up from the richly fertile coastal belt and the Carnatic plain. Most of this area is affected by the humid climate of south India and its rainfall is fairly evenly distributed throughout the year.

Village stock. Travelling eastwards from Akola where *Brucella* reactors in village cattle are first encountered [viz., 4 out of 46 (8 per cent) at Akola and Ellichpur], abortions are first reported around Nagpur itself. Hereabouts. 12 out of 65 (18 per cent) animals tested were *Brucella* positive. South-east of Nagpur in the Chanda district abortions and positive reactors have been noted, whilst between Nagpur and Raipur and around Raipur itself abortions become even commoner and out of 71 animals 18 (25 per cent) reactors were found. A gap occurs in the survey south of Chanda and the enquiry is taken up again around Warangal in Hyderabad State, where, in at least three villages, abortions have been frequent in the last decade and the incidence of positive reactors was 42 per cent of the herds.

The territory just described merges with the coastal district of Orissa and the occurrence of village Brucellosis continues across the provincial boundary. Thus in the adjacent Orian district of Nawapara 42 per cent reactors were encountered in tests of several village herds and abortion was alleged to be very common. Around Sambhalpur, Jharsaguda and Angul the reactor rate was 31 per cent, whilst south of Cuttack in the Berhampur district the incidence of reactors was the highest yet recorded in villages (viz., 50 per cent) and abortions were again very common. Two *Brucella* A/M type strains (the term A/M is used from now on to define the aberrant village *Brucella* type found in south India and described on page 144) have been isolated from this area. In the recently opened up Orian interior round Russelkonda and Koraput positive reactors, while present, were much less frequent than in other parts. Passing south along the coast Brucellosis is again encountered at Vizagapatam; whilst at Bezwada, although only doubtful *Brucella* reactors have been so far obtained, abortion and enlarged synovial joints are reported. However very few tests in cattle have been made in this section. In Nellore town, only goats were available for bleeding, but an 8 per cent incidence of reactors in these animals suggests that Brucellosis is common in cattle hereabouts; a supposition corroborated by the results obtained in villages some 22 miles to the south, near Godur. Here, in one village, 16 out of 37 (43 per cent) cattle were strongly positive and clinical abortions were said to be very common. Three village strains were isolated in this area, once again all being of the A/M type. As yet serious infection has not been discovered around Madras city, but the disease has been diagnozed southwards along the coast at Chidambaram. The scene of the survey now shifts inland to the country around Trichinopoly, where infrequent tests failed to reveal infection. A little to the south, however, in Madura district, the trail was again picked up and infection was detected at the slaughter-house in Kodaikanal; whilst around Periaculum considerable infection was once more encountered. In eight villages hereabouts, 18 out of 71 (25 per cent) animals were positive to the test and abortion and enlargements of the synovial joints were common. Some infection was found in and around Madura itself. South and east of Madura, infection has been diagnosed near Tinnevelly and Ramnad respectively. Proceeding north and west from Madura, infection was again encountered at Pollachi whilst around Conoor and Ootacamund it is fairly widespread. In the Ootacamund slaughter house the incidence of reactors was about 8 per cent and in that of Conoor about 5 per cent, whilst in seven adjacent villages 22 (58 per cent) out of 38 cattle were positive to the test and abortion is alleged to be frequent. On the west coast, infection has been diagnozed in random slaughter-house tests at Calicut and Tellicherry. On the Mysore-Madras boundary, two more *taluqs* were found to be heavily infected viz., Hosur and Kollegal. In seven villages of the former *taluq*, 28 (55 per cent) out of 51 cattle were *Brucella* positive and in nine villages of Kollegal, 32 (35 per cent) out of 91 animals were reactors. Clinical abortion is said to be common in this area.

In Mysore State the occurrence of Brucellosis seems to be very much on the wane and on that side of the state adjacent to Kollegal, it was not possible to trace any infection, whilst the wild Amrit Mahal herds also appear to be disease free. One sharp outbreak has been detected, however, in a village of the Kadur district of northern Mysore, but notwithstanding that further north still village infection appears to die out with a few random weak reactors to be found in the Kanara forest in the extreme south of Bombay Presidency, a *Brucella* A/M type strain similar in character to the village strains of this region has been isolated from a synovial enlargement of a cow of the Kanara district.

It must be remarked that most of the positive sera collected in Madras reacted to a high titre and one or two reached to the unusual orders of 1 in 10 and 20 thousand.

Although the diagnostic value of the serum agglutination test for Brucellosis is discussed fully in the appropriate place, it might not be out of place in view of the peculiarities of village survey and the large number of positive reactors obtained in this region, to give here a Table showing the incidence of reactors in relation to the clinical history of the animal, as alleged by the village spokesman. Table (VIII).

TABLE VIII

The relation between Brucella *reactors and disease in south-Indian village cattle*

Serum reaction	Abortion	Synovial enlargements	Retention of the placenta	Sterility	Healthy	Totals
Positive	113	38	1	18	32	202
Doubtful	14	7	4	8	32	65
Negative	59	39	9	92	274	473
TOTAL .	186	84	14	118	338	740

Percentage of aborters positive . . . . . 66 per cent
Percentage of synovial enlargement cases positive . . 49 per cent
Percentage of retention of placenta cases positive . . 10 per cent } Doubtful reactors being ignored.
Percentage of steriles positive . . . . . 16 per cent
Percentage of healthies positive . . . . . 10 per cent

Such a Table is of course a very rough guide, as conditions vary greatly from village to village. For example, whenever numerous abortions were reported in a small village, several positive reactors were nearly always found amongst them ; but when only one or two aborters were to be found these animals would often be negative and such isolated cases make up a large number of the 59 negatives shown in the Table. It would appear that synovial enlargements are more often than not connected with Brucellosis, as many of the negative animals included in the Table had passed the active stage of fluid enlargement and had reached the stage of induration, when animals are less likely to react.

The number of villages considered in the foregoing review was approximately 70.

Reports of abortions in sheep and goats are very unusual throughout the whole of this region and the over-all incidence of *Brucella* reactors in these animals was 3·1 per cent, the greatest incidence being found around Nellore and in Orissa. At Vizagapattam, of 892 sheep and goats, but 15 (less than 2 per cent) were positive.

Farm stock. In the indigenous stock of Government and jail herds Brucellosis was as a rule absent. One exception was a government farm on the east coast, where abortions in cows and buffaloes occasionally occur ; seven out of 55 animals were positive to the *Brucella* test. At one time there was an infected military herd of cross-bred cattle near Conoor, which has since been dispersed. The remnants of this herd are still to be found at Bangalore, but the disease does not appear to have spread from them to co-habiting indigenous cattle. A private herd of mixed European and indigenous cattle at Mysore is also infected.

Region 6. Central and northern Bombay and adjacent states

This region is bordered on the west by a fertile coastal plain, wide in the north, but narrowing to the south, between the western ghats and the sea. The major portion of the interior is a plateau, or hilly. Apart from June to October, when in the coastal tract the monsoon is exceptionally heavy and is elsewhere considerable, the northern portions and interior are, for the rest of the year, practically without rain. The southern extremity however, receives more than 2 in. of rain during March, April and May. Rainfall is, therefore, bi-seasonal at this spot only.

Village stock. As far as can be ascertained Brucellosis in village cattle is at present rare in this region. It seems probable, however, that, in the coastal belt at least, infection may at any time spread to the villages from the severe epidemics sometimes found on Bombay farms.

Organized farms. Most of the organized farms of this region lie in the coastal tract adjacent to Bombay and many of them are private gowshalas, some of which have developed into quasi-commercial concerns. Some carry indigenous cows whilst others combine these with buffaloes. There are also a number of dairies in and around Bombay that keep in-milk buffaloes only.

There are four military farms carrying cross-bred stock in this region proper and another closeby in the Central Indian Agency. In the four smaller farms the abortion rate is less than 2 per cent per annum and the incidence of *Brucella* reactors is 12 per cent alike of tested buffaloes and cows. In the fifth and largest farm, the annual abortion rate was 8 per cent of cows and 4 per cent of buffaloes, while the incidence of *Brucella* reactors at the time of inspection was 41 per cent and 63 per cent respectively. The former figure is surprisingly low and the latter, the highest on record for Indian buffaloes.

But two farms of indigenous cows are believed to be free from Brucellosis, one a government herd of Amrit Mahals at Bankapur and another a private herd of Gir cattle about 20 miles outside Bombay city. In the remaining three herds, visited in 1940-41, abortions were occurring, although their histories were unrecorded or confused, while, despite the great difficulty of obtaining permission to bleed, positive *Brucella* reactors were found. The average abortion rate in these herds was 1 per cent per annum and positive reactors occurred at the rate of 34 per cent of animals bled. Since 1940, severe epidemics of abortion have been found in two additional herds of this type; in one, 77 per cent of the breeding stock aborted in two years and 95 per cent of the aborting animals were *Brucella* positive; in the other, 40 animals aborted in a few months. Both farms purchased from Bombay city, pregnant animals which on abortion started the epidemics.

Once there were several buffalo dry-stock farms lying along the humid coastal belt, north of Bombay and serving the milking stables of that city, but all have since been closed owing, it is alleged, to the very great incidence—said to be 50 per cent of abortion in their stock. Abortion in the numerous Bombay city milking stables is, on the other hand, rare, because down-calving animals are brought there directly from Gujrat and the Punjab and only remain for the period of profitable lactation during which time most are non-pregnant. The incidence of reactors in some 126 empty milking animals from these stables was 5·5 per cent; but in 37 pregnant animals, that had been served in Bombay and were being sent away for the dry period, the rate had increased to 10 per cent. The incidence of abortion in the most important permanent buffalo farm in the humid coastal tract was 21 per cent for the years 1936 to 1940; a major endemic. Before 1936, the annual abortion rate was 3·5 per cent, but in that year infection seems to have been introduced by the purchase of 16 dry buffaloes from Bombay city stables, 11 of which aborted. In 1940 the incidence of blood reactors on this farm was 50 and 15 per cent of aborting and non-aborting animals respectively.

One or two buffalo farms, situated in the drier hinterland, have suffered minor epidemics of abortion, and one outbreak can be definitely related to the purchase of animals from Bombay stables. On the whole, therefore, it appears that many, though perhaps not all, Bombay stables are hotbeds of infection and that the dispersion of pregnant animals therefrom is a cause of major and minor epidemics or endemics elsewhere in the province. At one time, this conclusion was thought to concern the buffalo trade only, but it has since transpired that cows also can be incriminated.

SECTION II (EPIDEMIOLOGY).—COMPARATIVE SUSCEPTIBILITY

Cows

To compare the very rare and well cared-for European cattle of India with the more plentiful cross-bred or the superabundant zebu would be inequitable and in any case there are insufficient records so to do. Therefore, unless otherwise stated, European and not Indian figures relating to European-blooded cattle are given in the Tables to follow.

European-zebu cross-breds also form a class apart, in as much as almost all occur on military farms, where they are benefitted by a scientific husbandry, including an excellent order of housing and hygiene. In one way, this is a convenience because all can be placed in a single class, but in another, it is a disadvantage, as it is not possible to estimate their susceptibility when ill-nourished and badly housed; they must, therefore, be compared cautiously with certain classes of zebu. It is to be noted also that most cross-breds co-habit * with zebu or buffaloes, or both.

Recorded indigenous cows fall into one very large and one quite small group; so that here again comparisons are statistically unfavourable. The larger group consists of zebu living alone or co-habiting with other indigenous species. The smaller group is composed of zebu co-habiting with European crosses. The groups are so distinguished because there is evidence to show that the former acquires Brucellosis less readily than the latter.

A collection of data concerning the comparative susceptibility of the above classes appears in Tables IX-XIV. In the main, figures have been collected from herds scattered throughout India and because, as a rule, not more than one visit to each farm was possible, they are derived from a single random enquiry only.

As far as comparative susceptibility is concerned, Tables XI and XII are not specially informative; they are included merely for reference. Tables IX and X, on the other hand, suggest that, in comparison with European experience, cross-bred are nearly as susceptible as pure Europeans, but that the non-cohabiting zebu is much more resistant. As might be inferred from their greater exposure to infection, co-habiting zebu appear to be more resistant than cross-breds, but less so than zebu living alone.

These conclusions are supported by additional data in Tables XIII and XIV.

TABLE IX

The mean incidence of abortion amongst various classes of cow stock

Class	No. of herds observed	Population observed	No. of †aborters observed	Incidence per cen
European-zebu crosses	15	1337	273	20·4
Indian zebu, co-habiting with crosses	13	164	12	7·3
Indian zebu, not co-habiting with crosses	11	4495	167	3·7

* This term is used throughout to mean 'occupy the same premises'.

† Aborters = animals with history of abortion at the time of a single random observation.

TABLE X

The occurrence of repeated abortion amongst various classes of cows

Class	Population observed	Abortion				Mean expectation of calf loss	
		Once Per cent	Twice Per cent	Thrice Per cent	Four times Per cent	(*a*) *Per aborting cow	(*b*) †Per lifetime of 100 random stock
European	..	68·70	..	..	..	..	..
European-zebu, crosses	309	71·2	25·2	3·2	0·4	1·3	26·5
Indian-zebu, co-habiting with crosses	8	75·0	12	1·2	..	1·3	9·5
Indian-zebu, not co-habiting with crosses	134	94	5	1	..	1·07	3·9

* Calculated from actual recurrence of abortions in the populations observed.

† The figure obtained in* multiplied by the average incidence of abortion per cent.

TABLE XI

The pregnancy at which abortions occur in various classes of cows

Class	Pregnancy												Population observed.
	1	2	3	4	5	6	7	8	9	10	11	12	
European-zebu crosses.	34	27	12	9	8	2		less than 1 per cent					228
Non-co-habiting zebu	46	26	13	9	3	2		less than 1 per cent					180

Figures = abortions per cent, occurring at the pregnancy shown.

TABLE XII

The age at which cow-foetus are aborted

Class	Population observed	Age of foetus in months					
		3	4	5	6	7	8
European	95	5	6	14	15	24	31
European-zebu crosses	102	4	8	13	22	26	29

Figures = percentage abortions occurring at the months shown.

TABLE XIII

** The relation between the occurrence of cross-bred animals and the incidence of Brucellosis in seven herds of cattle*

Farm	Cross-breds	Brucella reactors	Incidence per cent of		
			Aborters† all breeds	Aborters cross-breds	Aborters country-breds
I	90	50	42	45	20
II	82	45	25	27	16
III	80	39	30	33	17
IV	42	11	11	9	12
V	17	24	10	4	11
VI	1	13	10	..	10
Means .	52	20	21	20	14

* Taken from records of the Imperial Veterinary Research Institute on contagious abortion from 1925 to 1935.
†The term 'aborters' refers to animals with a history of abortion which are present in a herd at the time of a single random observation.

TABLE XIV

The abortion rate in various grades of cross-breds *

Class of animal	Population	Number of aborters	Abortions Per cent
Fresian (Indian records)	18†	2†	11·1
7/8 grade	108†	8†	7·3
3/4 ,, ...	329	21	6·4
5/8 ,, ...	152	9	5·8
1/2 ,, ...	464	14	3·0
1/4 ,, ...	134	2	1·5
Indigenous	275	4	1·4
Mean annual abortion rate total population=3·3 per cent.			

* Taken from the annual reports of the Northern circle of Military Dairy Farms, 1935 to 1938, by kind permission of the Director of Military Farms, General Headquarters, Quartermaster General's Branch, Simla.
† Mean of three years; the remaining figures mean of 4 years.

An attempt has been made to give some comparative measure of susceptibility and in this connection column 8 of Table X requires some explanation. The figures given here are an expression of the mean expectation of loss of calves due to abortion, during the working lifetime of 100

random animals. They are obtained by multiplying the mean expectation of loss of calves per aborting animal [Table X column 7] by the mean expectation of unrepeated abortion during the lifetime of 100 random animals [Table IX]. If the smallest figure is taken as unity, the relative susceptibility of zebu, co-habiting zebu, and crosses is found to be as 1 : 2·5 : 7. In future, figures so obtained will be alluded to as the susceptibility index the figure for the zebu being, in all cases, regarded as unity.

Some findings suggest, either that the susceptibility of Indian cows to Brucellosis is decreasing, or that a better knowledge of the disease in relation to animal management is limiting its spread. For example, the reports of the military farms (northern circle) from 1932 to 1939 show a progressive annual decrease in the abortion rate ; a decrease which runs parallel with improvements in housing and management. On the other hand, since 1939 there have been indications that, in the busier farms at least, the rate has again increased and this has coincided with the unavoidable lessened attention and over-crowding due to the wartime emergency and expansion. It seems probable, therefore, that good management, rather than reduced susceptibility, was the cause of the earlier decrease ; nevertheless—although this seems the less likely explanation—the whole episode may be the result of phasic variations in susceptibility. Another illustration of decreasing incidence of Brucellosis is to be found in the Punjab Grantee farms. In the year 1923 the mean abortion rate, calculated from a single random observation of five of these farms, was 3 per cent of 1264 animals, whilst at later visits, in 1939, the figure had fallen to 1 per cent of 835 animals.

It must be noted that all the animals considered in the foregoing two paragraphs are kept under somewhat similar circumstances and that their standards of life are good, many of the pure-bred zebu herds in particular, being kept under semi-ranch conditions, in a favourable climate.

Exceptionally severe epidemics of abortion occasionally occur, even in non-cohabiting zebu and Tables XV and XVI give data about one such outbreak. The farm in question lies in a favourable climate and was formerly a typical Pinjrapole. Lately, however, it has been developed commercially and better stock was purchased, unfortunately from Bombay city dealers. Bombay city is a reservoir of Brucellosis and, as in other cases, this epidemic appears to have been introduced by the abortion of these purchases. Unhappily no records are kept in such institutions and the reliability of this data is dependent on the memory of the stockman, whose statements, nevertheless, were sufficiently supported by test and cross-enquiry to be credible. If in these circumstances the annual abortion rate during the period of this epidemic may be assessed at a conservative 25 per cent, the susceptibility index of this herd works out at 11·1, or nearly double the average for cross-breds. That the abortion was due to Brucellosis, seems to be proved by the fact that 95 per cent of the aborted animals were strongly *Brucella* positive.

TABLE XV

The abortion rate during exceptional Brucella *epidemic among indigenous cows*

Year	Total breeding stock (approximate)	Number of aborters	Abortions Per cent
1941	50	16	32
1942	50	9	18
Not known, believed 1942	50	16	32

Breeding herd total at the time of observation 1943	52
Total aborters at the time of observation	40
Total aborters per cent at the time of observation	77

TABLE XVI

The incidence of repeated abortions during an exceptional Brucella *epidemic amongst indigenous cows*

	Numbers stated to have aborted			Mean expectation of calf loss	
	Once	twice	thrice	Per aborting cow	Per lifetime of 100 random animals
Actual	19	16	5	..	..
Per cent	49	40	12	1·65	41·25

In the absence of records, it is impossible to give figures for the comparative susceptibility of village cows, but it seems probable that they do not differ much from other zebu.

Buffaloes

Abortion figures for buffaloes are given in Tables XVII to XXII and their susceptibility index works out at 1·5. Thus, on this calculation, they are rather more susceptible than non-cohabiting zebu (index=1·0), while they are nearly twice as resistant as co-habiting zebu and over 4 times as resistant as crosses. It is to be noted, however, that the buffaloes to which these figures relate were, in fact, occupying the same farm, if not always the same sheds, as cross-bred cows ; they are, in effect, ' co-habiting ' buffaloes and it is to be supposed, therefore, that the non-cohabiting buffalo is in reality less susceptible than the non-cohabiting zebu.

TABLE XVII

The mean incidence of abortion amongst buffaloes

	Observed population	No. of farms	Incidence of aborters present at a single random observation	
			Actual	Per cent
Buffaloes	1,880	15	110	5

TABLE XVIII

The occurrence of repeated abortion amongst buffaloes

	Observed population	Abortion occurring			Mean expectation of calf loss	
		Once	Twice	Thrice	Per aborter	Per lifetime of 100 random animals
Actual	205	189	16	..	1·08	5·4
Per cent	100	92	8	..		

TABLE XIX

*The pregnancies * at which abortions most commonly occur in buffaloes*

Pregnancy	1	2	3	4	5	6	7	8	9	10	Population observed
Actual	21	51	23	14	9	9	3	Less than 1 per cent			130
Per cent	16	39	17	10	6	6	2	Less than 1 per cent			100

* As almost all farm-buffaloes are purchased as adults, figures of this Table refer to pregnancies recorded on the farm. Earlier pregnancies in village may have occurred.

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TABLE XX

The age at which buffalo-foetus are aborted

	Age of foetus in months							Population observed
	3	4	5	6	7	8	9	
Actual	3	3	8	14	22	26	27	103
Per cent	3	3	8	14	22	26	27	100

TABLE XXI

The annual abortion rate during an exceptional endemic among buffaloes

Year	Total pregnancies completed	Total abortions	Pregnancies abortions per cent
1936	117	34	29
1937	127	22	17
1938	138	37	27
1939	120	20	17
1940	155	39	25
1941	174	28	16
Means	138	30	21

TABLE XXII

The age at which foetus were aborted in an exceptional epidemic among buffaloes

	Age of foetus in months							Observed population
	3	4	5	6	7	8	9	
Actual	10	6	3	7	1	4	1	32
Per cent	31·3	18·1	9·4	21·9	3·1	12·5	3·1	100

12 per cent of this herd aborted within 6 months.

As with cows, so with buffaloes there are exceptional rates of abortion. These occur in two forms, *viz.*, (1) severe epidemics, of which Brucellosis is fairly plainly the cause and (2) exceptional epidemics appearing mostly in villages, about which little is known. The former are not very common, but data of two such epidemics are given in Tables XXI and XXII. The latter are discussed more fully on page 188.

Sheep and Goats

In the five years of the scheme not a single abortion in sheep has on investigation been attributable to Brucellosis, nor can the writer recall a significant incidence of reactors in these animals, either where abortions were epidemic, or where sheep were in contact with infected cattle. It must be supposed, therefore, that the susceptibility of Indian sheep to Brucellosis is indeed slight.

The goat, on the other hand, seems to be rather more susceptible, for, although Brucellosis has not hitherto been diagnosed in cases of clinical abortion amongst village goats, in two old-established goat farms, this disease has undeniably co-existed with abortions. The specificity of these abortions has, however, been disputed and their description has consequently been relegated to the section on non-specific abortion.

Whilst testing in slaughter houses, occasional isolated reactors have been encountered amongst sheep and goats alike and it is remarkable that these have been occurred only in south India, where Brucellosis is common in village cattle, *viz.*, of 405 sheep and goats bled in 15 towns of south India, 11 (2·5 per cent) were positive ; of 250 tested in 11 towns of north India, none was positive.

Inextricably entangled as it is with many improperly understood features of disease transmission susceptibility *per se* is a most imponderable characteristic and its mensuration on a basis of rates of abortion and loss of calves is consequently bound to be fraught with inaccuracies. Nevertheless, it is felt, that the foregoing are tolerable estimates of the ordinary liability of Indian cattle to contract Brucellosis. In an exceptional environment, any class of stock may behave in an unpredictable manner and the response of animals to infection by Brucellosis is not likely to be exempt from such irregularities.

One more point requires mention ; it has been stated in the past that many Punjab goats are infected with Brucellosis and the infection rate has been variably estimated at from 8 to 60 per cent. These rates are greatly at variance with themselves and the present findings, but it must be recalled, that the antigenic instability of rough *Brucella* strains was not understood until the middle-thirties of the present century and that the *Br. melitensis* strains, usually used for testing goats, were almost always rough. Numerous old records are still in existence, wherein 9/10 of the positive results of *Brucella* tests are clearly the outcome of unstable antigens and this supposedly would account for many former misconceptions. Infection in the goat will be referred to at greater length when the question of human infection is being considered (page 153).

SECTION III (EPIDEMIOLOGY). THE DISSEMINATION OF BRUCELLOSIS IN INDIA

The roles of climate and hygiene

The channels by which *Brucella* leave their host are too familiar to deserve discussion in so prescribed a space as this paper. The point to be noted is that, although, *Brucella* organisms are considered obligatory parasites, in favourable circumstances, they will survive and disseminate outside the host.

In this country, the effects on their survival of sunshine, of high temperatures and of dryness, on the one hand, and, of humidity, of moderate temperatures, and of reduced sunlight on the other, can be vividly contrasted in north and south India. In the whole of the north-west, scorching sunshine is diurnal from April to October, natural shade is rare, while the annual rainfall is but one inch, or less. In the south, conditions are reversed, temperatures vary little about the blood heat of cattle, the atmosphere and the land surface and soil are moist while, not only is natural shade everywhere

more common, but for fairly long periods the sun is overcast altogether. But it has already been shown that Brucellosis is endemic in the south and rare in the north-west (page 116). The simple and understandable inference is, that sunshine and dryness are inimical to *Brucella* microbes, or conversely that equible temperatures, humidity and shade are propitious to their survival.

Other more isolated observations corroborate these suppositions. On page 128 allusion has been made to the decrease of Brucellosis on the Punjab Grantee Farms. These farms lie in a semi-dessert tract and for the most part their indigenous stock live and calve out-of-doors. It is possible that this is an example of self-elimination of Brucellosis due to animals living in dry, sunlit surroundings. In contrast to this, are the returns from three farms situated in Assam, a region which endures a heavy and protracted monsoon and which, moreover, lies within the bi-seasonal rainfall belt of India. The animals of these farms are of similar type and origin to those of the grantee herds, yet in 1941 the incidence of reactors amongst them was 20 per cent, whilst their abortion rate is from 2 to 6 per cent per annum.

In another indigenous herd of the Punjab [farm (1)], the mean annual abortion rate during the climatically normal period 1930 to 1935 was 1·27 per cent of completed pregnancies, whilst, during the entirely rainless period 1936 to 1939, this rate was 0·66 per cent, *i.e.*, a reduction by half. Here, another important consideration arises, for, during the rainless years the stock could not be grazed and had to be stall-fed in congested corrals. The result of this over-crowding was a severe epidemic of tuberculosis. It appears, therefore, that conditions of congestion, sufficient to produce an air-born respiratory epidemic of a relatively resistant organism, resulted in a diminution of *Brucella* infection during a period of constant sunshine and extreme dryness. Congestion as a factor in the spread of Brucellosis, therefore, may be secondary to excess of sunlight and it seems likely that Brucellosis is essentially not an air-born disease.

In contradistinction to this result, are the findings at a pinjrapole [farm (2)] situated on the arid Deccan plateau. During 1941-42 this farm suffered an extremely violent epidemic of Brucellosis and 77 per cent of the herd aborted. Here, climatic variation was negligible and sunlight was more or less continuous. Congestion also was extreme, the animals never being out of the farm compound. The contradictory results of farms I and II may perhaps be explained on the grounds of hygiene. In farm (I) aborting animals are segregated and gross infective materials, such as the placenta, are quickly destroyed. In farm (II), no such precautions were taken and general cleanliness was lacking.

In cows, therefore, it appears that, provided gross sources of dissemination are removed, the sterilizing effects of sunlight are sufficient to suppress the remaining minor sources of infection, even when congestion is great; conversely, where animals are dispersed hygiene becomes of less importance and the effects of sunlight are paramount. Village cattle, on the whole, graze over wide areas and bacterial dispersion, therefore, counteracts the lack of hygiene, so that, here again, sunlight plays a major part in the suppression of contamination.

There are, however, other aspects of the effects of climate upon the dissemination of *Brucella*, *i.e.*, humidity and rainfall and the possible role played by moisture is very well illustrated by the restriction of indigenous Brucellosis to the humid tracts of India, and especially by some aspects *Brucella* epidemiology in buffaloes. Only one serious endemic of Brucellosis has been encountered in buffaloes (farm 3). The abortion rate on this farm is shown in Table XXI and, for India, it is undeniably excessive, possibly unique. In an attempt to explain this unusual incidence, many environmental factors have been considered, but the only extraordinary influence that could be found was the exceptionally heavy monsoon, amounting to 80 in. of rainfall in the period June to September. Table XXIII presents a statement of the percentage rates of abortion amongst susceptible stock for each month of the year, the calculation being made from an aggregate of the returns for five years. The head of stock that was adjudged susceptible to abortion in any one month was the aggregate of animals carrying a foetus between five and nine months of age inclusively. This period of term was selected after a study of Table XX. A further study of the same Table, however indicates that the liability to abort differs in the various months selected in the proportion of

1 : 1·75 : 2·75 : 3·25 : 3·5 for the months five to nine respectively. In each case, therefore, the aggregate of animals pregnant in each month was multiplied by its respective liability factor, and the sum of these calculations was taken to be the corrected number of susceptible animals [Table (XXIII) column 8].

TABLE XXIII

The monthly incidence of abortion in the buffaloes of farm (3)

Month	Term of pregnancy in months and the abortion liability ratio for each month					No. of abortions aggregate for 5 years	No. of susceptible animals	Ratio of column 7 to 8 per cent
	9 (3·5)	8 (3·25)	7 (2·75)	6 (1·75)	5 (1·0)			
January . . .	12	7	11	10	10	2	124	1·6
February . . .	7	11	10	10	46	3	154	1·9
March	11	10	10	46	131	4	314	1·2
April . . .	10	10	46	131	134	10	567	1·7
May	10	46	131	134	116	14	909	1·5
June	46	131	134	116	80	17	1,021	1·6
July	131	134	116	80	40	14	1,377	1·1
August	134	116	80	40	21	27	1,184	2·3
September . . .	116	80	40	21	12	36	861	4·2
October . . .	80	40	21	12	7	21	517	4·1
November . . .	40	21	12	7	11	5	269	1·8
December . . .	21	12	7	11	10	1	162	0·6

Figures in columns 2 to 6=the number of animals pregnant at the term shown, in aggregate for 5 years.
Figures in column 8=the sum of the figures in columns 2 to 6, after application of the abortion liability ratio, *plus* the figures in column 7.

The result of this calculation shows that the abortion rate for the months November to July varied little about a mean figure of 1·4. It is further to be noticed that, although the population of susceptible animals steadily increases from January to July, there is no corresponding increase in the abortion rate. In August, however, whilst the number of susceptible animals commences to decrease the abortion rate suddenly doubles and in September and October it is just thrice the mean for the normal months of the year. Thereafter, the rate returns to normal. Now as the period of transmission and incubation of the disease is likely to occupy a period of one to two months, the critical period of increased infection rate causing the increased abortion rate during August, September and October, commences in mid-June and lasts up to August. But in this period the abortion rate and, therefore, presumably the bacterial elimination rate, is constant, whilst the corrected number of susceptible animals does not vary greatly round a mean of 1200 ; further the absolute number of abortions in July is the same as in May. In other words donor and receptor influences do not vary sufficiently during this period to account for the large increase of abortions in August, September and October, and this increase, must be explained by a change in environment which affects bacterial survival and transmission. But from June until August the climate changes completely from diurnal sunlight to total sunlessness and continuous rainfall and this suggests that either the absence of sunlight, or the presence of moisture, or both, favours the spread of Brucellosis in buffaloes.

As before, however, the factor of congestion complicates this picture. During the monsoon buffaloes cannot be grazed and they are consequently standing in congested cowsheds, sometimes for weeks at a stretch. The influence of congestion may perhaps be eliminated by comparing this result with the records of farm 2, page 131, which, besides carrying the herd of *Brucella*-infected cattle already discussed, keeps a herd of closely co-habiting buffaloes. Now on each farm, the factor of congestion is probably about equal but on farm 2, as evidenced by the spread in cows, the abundant sunlight was unable to destroy the infection. Thus the two environments may be compared as follows :—

Environment	Farm 2	Farm 3
Climate	Sunny and dry	Overcast and humid
Disposal of animals	Congested	Congested
Source of infection	Very great	Ample
Result	No epidemic	Pandemic

The above data, surely suggest that, in buffaloes at least, rainfall plays a considerable part in the dissemination of Brucellosis.

Without wishing to labour the point several other *Brucella* epidemics in buffaloes offer supporting evidence. Another dairy farm (4), situated in a dry healthy climate, purchased several buffaloes from a humid, *Brucella*-infected locality. These animals aborted shortly after arrival, but up-to-date there has been no rapid spread of Brucellosis to the remainder of the herd. This record holds an entirely new significance, for, it is possible to suppose that the buffalo requires for its infection a strain of *Brucella* that has been adapted to these animals by passage—a hypothesis which might account for the failure of transmission in farm 2. But in this case buffaloes themselves are bringing a presumably adapted strain to a disease-free herd and yet transmission does not occur.

In another farm (5), there was a major epidemic of Brucellosis in cows during the normally dry summer of 1940, whilst the abortion rate in a co-habiting herd of buffaloes remained average for their class. In July 1942, however, there was an exceptional monsoon, which coincided with a second major epidemic in cows and which was immediately succeeded by a major epidemic in buffaloes. The inference, here, is self-evident. The year 1942, moreover, was remarkable for a heavy monsoon throughout India, and several reports were received of abortion epidemics amongst Punjab village buffaloes ; in some cases these outbreaks were associated with the rains. Another report was received from a private farm in charge of an American veterinarian, who, unprompted by the writer, stated that the buffalo abortion was associated with the exceptional rainfall.

These records all lead to the same conclusion and it is tempting to accept the simple explanation that in disseminating the disease amongst buffaloes, at least, a fluid vehicle is more satisfactory than a dry one. In this respect the buffalo's habit of wallowing requires consideration, and, at first sight, it might seem to be the key to the problem. On closer investigation, however, it appears that the period of the year when it is essential for buffaloes to wallow is during the dry heat of April, May and June, at which time water is everywhere short and the few available small tanks and ponds are grossly congested with animals. Later, during the rains, wallowing is still widely practised, but the animals are dispersed over a far wider expanse of water. If, therefore, the fluid of the wallow is the vehicle of transmission *Brucella* epidemics would be worse in dry summers when the liquid would be most concentrated, whilst, assuming a one or two month transmission and incubation period, abortion epidemics should commence in May or June rather than August, or September.

It seems, then, that the vehicular action of water may not be the explanation. It is possible for instance that the rainfall and restriction of animal movements may result in a loss of resistance, a supposition which is in part supported by the buffalo's peculiar liability to abort as the result of

systemic disturbances. More remotely, it may be supposed that many buffalo abortions are not due to Brucellosis at all, but to some quite unrecognized factor of rainfall, a preposition which receives further consideration on page 188.

Plainly, the dissemination of *Brucella* in India is influenced by several interdependent factors and, while it is not possible wholly to disentangle their individual roles, some tentative postulates come to mind. The factors which inhibit fruitful dissemination are elementary hygiene, sunshine and animal dispersion, and where indigenous cattle are concerned, the optimum action of all the three will result in a gradual elimination of the disease, or prevent its gaining a hold on an already clean farm. Further, the combined action of any two factors is likely to minimize the disease, but, where one factor alone is operative, the disease will in all probability spread. Conversely, the influences propitious to dissemination are absence of hygiene, absence of sunshine and congestion.

The conflicting roles of humidity and dryness are less ponderable, but at least it is easier to clean dry hard surfaces, than wet and muddy ones, while the vehicular value of fluids cannot be ignored.

Many epidemiologists have associated moisture with the spread of diseases. Rogers [1926], for example, found that cholera spreads as the absolute humidity reaches 0·4 in. and it may be noted in passing that, in lower Bengal and on the Coromandel and Malabar coasts (*i.e.*, in the *Brucella* endemic areas of India), the absolute humidity is never below 0·4 in.

About *Brucella* little has been proffered, but Horrocks [1905] found that these organisms survived for 43 days, in soil that was allowed to dry naturally (in Malta) and for 72 days in damp soil. Be that as it may, whether soil-moisture, the relative or absolute humidity of the atmosphere, or a tri-seasonal rather than uni-seasonal rainfall is considered, the main function of the moisture will be to delay or obviate drying. Consequently, the effect of drying *Brucella* has been studied in one or two simple experiments.

In one series of tests, 0·5 cc. of a *Brucella* suspension in saline (density=Brown's scale 5 ; approximate live count=10,000 million organisms) was pipetted on to filter papers previously placed in lightly plugged test tubes. A 1/10 dilution of the suspension could be reconstructed from these papers by pipetting 4·5 cc. of saline into a still-wet filter paper, or 5·0 cc. into a paper that had dried. Re-suspension was effected by pulping and wringing the paper with the pipette. The resulting suspension was used to make a ten-fold rising dilution series for counting. Later, this technique was abandoned, as unevenness of the paper pulp caused irregularity of counts.

Using this method, the effect of rapid drying *in vacuo* was studied. Attached to a Chryochem* apparatus and subjected to a vacuum of 50μ the papers took about 1 hour to dry. No artificial freezing was used but self-freezing may have occurred towards the end of the process. The suspension was counted before placing on filter paper, again after reconstruction immediately after deposition on the paper and finally immediately after drying. The results of duplicate counts are shown in Table XXIV and reveal that a large number of *Brucella* die during rapid drying under vacuum, even at room temperature (20-25°C).

TABLE XXIV

The survival of Brucella *during rapid drying (Chryochem apparatus)*

Nature of suspension	Count in millions per cc.	
	Trial I	Trial II
Saline suspension at the time of preparation	6,300	4,400
Suspension reconstructed from wet filter paper immediately after wetting	3,000	3,800
Suspension reconstructed from filter paper immediately after drying	150	190

* (Flosdorf-Mudd (1938) method) F. J. Stoks Machine Co., Philadelphia, U. S. A.

After drying, the vacuum was released and some unused papers were stored over $CaCl_2$ at 42°C; from time to time they were pulped and the suspensions counted. Despite the irregularities already mentioned, the counts indicated that the death rate was dramatically swift (Fig. 1).

In a second experiment, using the same technique the artificial drying in vacuum was omitted and the samples were placed directly in the 42°C incubator, some over $CaCl_2$ and others over H_2O and H_2SO_2 variously mixed to produce relative atmospheric humidities of 40 per cent, 80 per cent and 90 per cent. This experiment failed because the death-rate was so rapid that it quite outstripped the dilutions selected for counts, all that could be learnt was that the samples over $CaCl_2$ showed a count of nil in the 1/100 and 1/10 dilution at 24 and 38 hours respectively (lower plates were not made). In the 90 per cent humidity, on the other hand, one count of 1·4 millions per cc. was obtained at the 56 hour.

It is to be noted that in the $CaCl_2$ atmosphere the papers were nearly dry at 20 hours, when a count of 800 million was obtained. Whereas, at 32 hours the paper was perfectly dry and in the interim the count had fallen almost to nothing. Conversely, the paper in the 90 per cent humidity was not dry at the 92 hour.

These preliminaries suggest that in the quite ordinary summer plains temperature of 42°C *Brucella* die within 36 hours, in dry air but in moist, remain alive for three or four days.

The foregoing technique was now abandoned and instead, 0·5 cc. amounts of suspension were pipetted into several covered, empty, 1¾ in. petri dishes, which in turn were placed in the various atmospheres. A suspension was reconstructed by adding 5·0 cc. of saline to a dry plate, whilst an estimated quantity between 4·5 and 5·0 cc. was added to one partially dry. (The error necessarily occurring here is appreciated, but, as such large differences are concerned it is probably not important.) Again, as 42°C is rather higher than the usual day maximum in the humid parts of India, the storage temperature was reduced to 37°C which is commonplace in winter and summer in the endemic areas. Using this method, it was found that 0·5 cc. of suspension over $CaCl_2$ dried completely between the 26 and 30 hour and that during the period 18 to 42 hours the count dropped from 990 millions to 0·066 million. In 80 per cent humidity on the other hand, the count dropped from 5,800 millions to 220 millions in the same period, while drying was incomplete at 90 hours (Fig. 2).

The above experiment was, nevertheless, unsatisfactory, mainly owing to incorrect estimates of which dilutions to plate at the later counts. Again it was thought that the salt in the saline suspension might be playing an inimical part as it became concentrated during drying. So the experiment was repeated but bacteria suspended in distilled water, instead of saline were used. The results appear in Fig. 3 and, while both death-rates are considerably slower in pure water than in saline, the mortality in dry atmosphere is again more rapid than in the humid. It is to be noted moreover, that natural drying of *Brucella* is unlikely to occur often in a substrate entirely free from salts.

The foregoing experiments contribute towards a better knowledge of the roles played by humidity and dryness in the dissemination of *Brucella* in Asia and reviewing them in conjunction with the actual findings of the Indian field, it might not be too rash to suggest that a cornerstone has been laid in the construction of a hitherto neglected aspect of *Brucella* epidemiology.

The role of seasonal breeding

When the buffaloes' habit of breeding seasonally causes them to be advanced in pregnancy during the monsoon, Brucellosis, once established, will tend to spread. This tendency might possibly be corrected by inducing the animals to breed at a more favourable season.

Reservoirs of infection

The principal reservoirs of Brucellosis in India are to be found where the environment favours dissemination viz., (i) in congested city stables, as found in Calcutta and Bombay, whence the disease

is disseminated by the sale of dry cattle to the surrounding country, (ii) in south-Indian villages; here, cattle movements from south to north are probably insufficient to permit a grave extension of infection, but a possible route has been suggested on page 145.

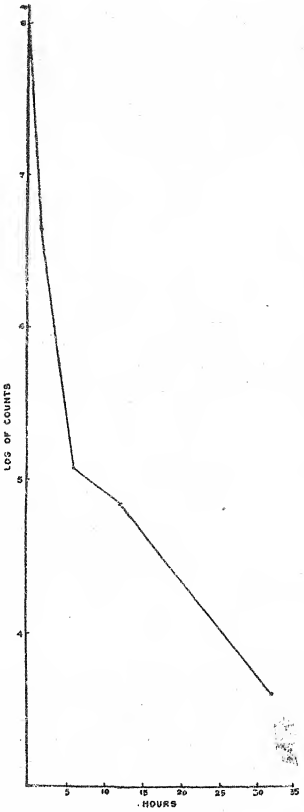

FIG. 1.The death rate of *Brucella* stored at 42°C. over $CaCl_2$, after having been dried on the 'chryochem' apparatus.

It is probable that a further big reservoir is even now developing. The vastly increased buffalo population in military establishments cannot be given the attention it would receive in normal times. Moreover, temporary farms are being erected in the unfavourable climates of south and east India. It is not improbable that Brucellosis will spread among these animals and these may disseminate disease to other parts of India when dispersed.

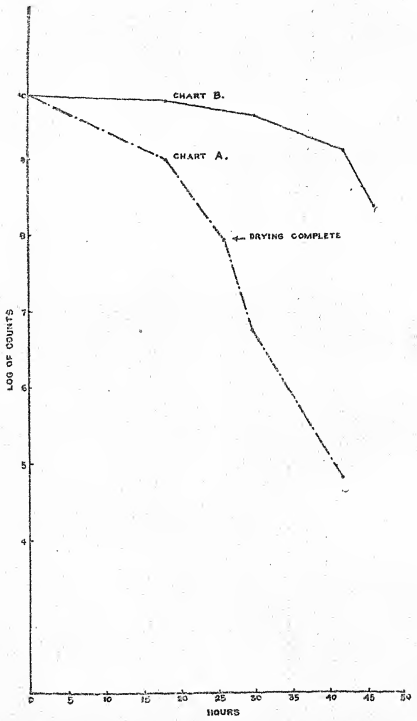

FIG. 2. The death rate of *Brucella* stored at 37°C.

A—over $CaCl_2$

B—in 80 per cent humidity.

(For table of counts see overleaf).

Table of counts for Fig. 2.

Hours	Count in millions	
	A. in dry air	B. in humid air
0	6700	6700
18	990	5800
24	..	..
26	9	..
30	0·58	3800
42	0·066	1280
46	..	220

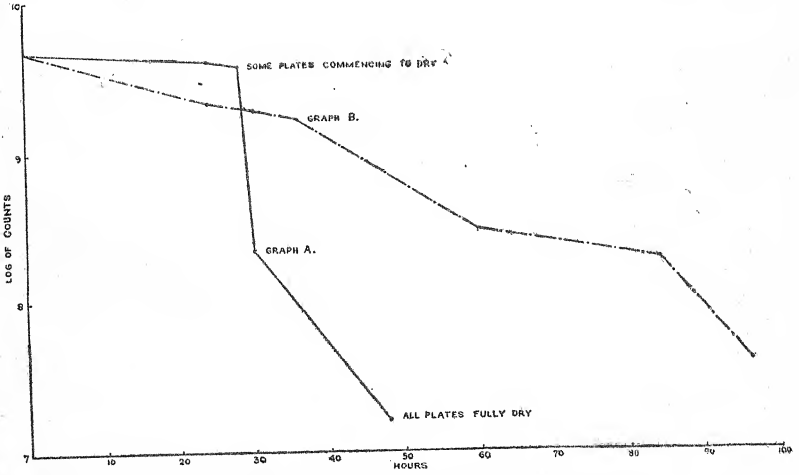

FIG. 3. The death-rate of *Brucella* (suspended in distilled water) stored at 37°C.

Graph A—over $CaCl_2$

Graph B—in 80 per cent humidity.

(For table of counts see overleaf.

D

Table of Counts for fig. 3

Hours	Count in millions.	
	A. dry air.	B. humid air.
0	4800	4800
24	4000	2200
28	3700	..
30	221	1940
36	..	1630
48	15	700
60	..	310
84	..	190
96	5	38

Section IV. The Bacteriology of Indian strains

To rehearse the whole bacteriology of Brucellosis, or to cite a torrent of references here, would be supererogatory and those unfamiliar with its details are referred to the many excellent treatise in the literature; in the pages to follow, the subject is dealt with solely in so far as it concerns the disease in India.

Classification

The bacterial genus *Brucella* is usually supposed to contain three species *Br. abortus*, *Br. melitensis* and *Br. suis*. Not all strains, however, can be so classified and, in an examination of some 2,000, Huddleson [1939] has mentioned 13 that were atypical. Some eight or ten methods of classifying the genus have been described, but the criteria most commonly used are based on (i) CO_2 requirements at isolation (ii) antigenic structure, (iii) H_2S excretion and (iv) the inhibitory action of dye media. Because all tests are relative estimations of biological processes and as most are very susceptible to errors of technique and personal judgement, they should be made as far as possible on a number of strains at the same time and by the same person, using a standard technique and a single batch of original media.

The techniques used in the present work have been as follows:—

(i) *CO_2 requirements at isolation.* All sowings for primary isolation of *Brucella* are incubated in a partial CO_2 atmosphere (cultures on solid media in 10 per cent and in fluid media 20 per cent of CO_2). At first subculture, all strains isolated, are sown in duplicate on liver agar slants pH 6·8, one slant being incubated in air and one in 10 per cent CO_2. Strains failing to grow in air are presumed to be *Br. abortus;* strains growing in CO_2 and air are taken as one of the two other variants.

(ii) *H_2S elimination.* When a sufficient number of recently isolated strains have accumulated, they, together with two strains of each of the three type variants, are sown into slants of tryptose agar prepared according to the maker's formula (liver agar may be used, but its sulphur content

is likely to vary more than that of tryptose). After sowing, a slip of filter paper, previously soaked in a saturated solution of lead acetate and sterilized in hot air, is inserted beside the plug of each culture-tube so that it projects into its lumen. The slants are incubated at 37°C for eight days. At the end of each 24 hours of this interval, the slips of filter paper are removed and fresh inserted. The removed slips are labelled, dated and retained as a record of the degree of blackening of their surface due to the formation of lead sulphide by the interaction of H_2S and lead acetate. *Br. melitensis* strains eliminate no H_2S. *Br. abortus* strains eliminate medium quantities of H_2S for three or four days only. *Br. suis* strains eliminate maximum quantities of H_2S for upwards of six consecutive days.

(iii) *The inhibitory action of dye media.* A single batch of tryptose or liver agar pH 6·6 to 6·8 is prepared and divided into seven lots. Lot (1) is left unaltered. Lots (2), (3) and (4) are melted and whilst warm a hot aqueous solution of basic fuchsin is added to each so that the final concentration of dye in the three lots is 1/75,000, 1/85,000 and 1/100,000, respectively. In the same way, a hot aqueous solution of thionin is added to lots (5), (6) and (7) so that the dye concentration in the final preparation is 1/50,000, 1/60,000 and 1/70,000 respectively. Plates are poured from each of the seven lots and two or three strains of each of the three type variants are sown into each of the seven different media. Seeding is done by transferring standard large loopsfull of a 48-hour-old broth culture of the strains under examination. The plates are incubated at 37°C for three days and their growth examined on the second and third day. The growth on the plain agar is taken as a standard for each strain and is compared with the degree of growth on the various batches of dye media. The object of this work is to select the concentration of dye which best permits a normal growth of *Br. melitensis* strains on both thionin and fuchsin and which at the same time, just inhibits the growth of *Br. suis* on fuchsin, whilst allowing its growth on thionin and inhibits the growth of *Br. abortus* on thionin whilst permitting its growth on fuchsin. Having selected the most suitable concentrations of dye, the unknown strains are seeded in the same way into plates of the selected concentration of dye media and plain liver agar. The inhibitory effects of the dyes are noted and the unknown strains allocated to their various types accordingly. In this work it is important that all media be freshly prepared and of the correct pH. No delay, therefore, should be permitted between the trial run with known strains and the tests on unknown strains.

(iv) *Antigenic structure.* Type specific antisera are prepared by injecting separate rabbits with one of the type variants, *Br. abortus* and *Br. melitensis*. One intravenous injection only is given the dose being approximately $4{,}000 \times 10^6$ organisms, in 1·0 cc. of saline. Sera with titres in the order of 1/2,560 are the most convenient in use and therefore the rabbit's blood must be tested daily after the third day and the animals killed and bled when the previous day's test shows a titre in the order of 1/640-1/1,280, or thereabouts. The sera should then be cold-stored for a week, during which time their titres will fall a little. To prepare the absorbing systems Wilson and Miles [1932] who also describe the theory of this work—recommend that the sera so obtained be diluted to 1/64 of their end-titres *e.g.* a serum with an end-titre of 1/2,560 would be diluted to 1/40 in saline. But, whilst this is a good rough guide, it is usually best to prepare three dilutions *viz.*, 1/32, 1/64 and 1/128 of the end-titre and perform a rough optimum proportion test. These three dilutions of each type specific sera are then used to wash off the surface growth of the heterologous *Brucella* strain from 48-hour-old agar cultures. In each case the previously diluted serum is added until the density of bacteria is approximately 7 to 8 on the Brown's scale. The resulting bacterial suspensions are incubated for three hours, their containers being shaken three or four times during this period. After incubation, the suspensions are centrifuged and the supernatent sera collected. The effect of absorption has been to remove the minor heterogenous antigen from each type specific serum and the *Br. abortus* serum has consequently become monospecific

abortus and the *Melitensis*, monospecific *melitensis* serum. Each primary dilution of each monospecific serum is then titrated against *abortus* and *melitensis* antigens, which are prepared in the ordinary manner (p. 149). If all has gone well, the monospecific sera should react to between the fourth and seventh dilution with their homologous antigens, and not at all, or to one or two dilutions only, with their heterologous antigens. In each case the dilution of monospecific serum which gives the greatest difference of titre between the homologous and heterologous titrations is selected for use. The series of dilutions used in the final titrations are prepared by doubling the dilution in each succeeding tube of a series and, assuming the primary dilution of a serum is 1/20, the final titration series would run 1/40, 1/80 and so on. Whilst, if absorption had been nearly optimum, the serum would react to about +1/640 with its homologous antigen and to about +1/40 with the heterologous. To differentiate unknown *Brucella* strains all that is necessary is to prepare antigens from them (p. 149) and titrate each against monospecific *abortus* and *melitensis* sera. It is to be noted that absorption of *Br. suis* antiserum is purposeless, as neither antibody is present in excess of the other. Similarly, if *Br. suis* antigen, in which neither antigenic factor is present in excess, be titrated against monospecific sera, equal titrations are likely to result. The rough or so-called 'para' strains cannot be classified serologically.

In India, the obtaining of satisfactory specimens for bacterial examination presents many peculiar difficulties not encountered in temperate climates. The main troubles are the early putrescene of dead tissues, dust contamination, long periods of transport in warm environment, but above all difficulties of communications, for abortions usually occur without notice in remote localities, which cannot be reached in time by persons qualified and equipped to take samples fit for bacterial examination. The *Brucella* strains it has been possible to secure have consequently been disappointingly few, but, such as they are, their origin and classification are given in Table XXV.

TABLE XXV

Classification of Indian Brucella *strains*

Locality	*Br. abortus*			*Br. melitensis*			Unclassified		
	Host			Host			Host		
	Farm cow	Farm buff.	Horse	Farm cow	Goat	Mare	Farm cow	Farm buff.	Village cow
Ahmednagar	1	0	1	0	0	0	0	0	0
Ambala	2	7	0	0	0	0	0	0	0
Bombay	1	2	0	0	0	0	0	0	1
Cuttack	0	0	0	0	0	0	0	0	1
Ghoom	1	0	0	0	0	0	0	0	0
Hissar	0	0	0	0	1	0	3	0	0
Jarsugudda	0	0	0	0	0	0	0	0	1
Jubbulpore	0	0	0	0	0	0	1	0	0
Kirkee	0	1	0	0	0	0	0	0	0

Locality	*Br. abortus*			*Br. melitensis*			Unclassified		
	Host			Host			Host		
	Farm cow	Farm buff.	Horse	Farm cow	Goat	Mare	Farm cow	Farm buff.	Village cow
Lahore	3	0	0	1	0	0	0	0	0
Lucknow	0	0	0	0	0	0	1	0	0
Meerut	0	1	0	0	0	0	0	0	0
Mukteswar	1	1	0	0	0	0	6	2	0
Mysore	1	0	0	0	0	0	0	0	0
Patna	0	0	0	0	0	0	1	0	0
Poona	0	0	0	0	0	1	0	0	0
Raipur (Madras)	0	0	0	0	0	0	0	0	3
Razmak	1	1	0	0	0	0	0	0	0
Sialkot	0	1	0	0	0	0	0	0	0
TOTAL	11	14	1	1	1	1	12	2	6

Total *Br. abortus*=26; total *Br. melitensis*=3; total unclassified=20.
Grand total=49 strains

In this classification, all 26 *Br. abortus* variants fell precisely into their class, with no discrepancies. The work was in all cases controlled by parallel tests on (1) European and American *abortus*, (2) Maltese *melitensis* and (3) American *suis* strains. It is noteworthy that all pure *abortus* strains come from organized farms most of which have carried, or still carry, European cross-bred stock, and they occur equally in cows and buffaloes. As regards the *melitensis* strains, in all the three, there was some doubt as to exact classification (Table XXVI).

TABLE XXVI
Classification of Indian melitensis *strains*

Strain	CO_2 requirements at isolation	Excretion of H_2S on successive days	Growth on		Agglutination against	
			thionin	fuschin	Mono-A serum	Mono-M serum
Abortus control	CO_2	+++ +++ ++ + +	..	+++	+++1/320	+1/20
Suis control	air/CO_2	+++ +++ +++ +++ +++ ++ ++ +	+++	+	0	0
Melitensis control	air/CO_2	*Nil*	+++	+++	+1/20	+++1/640
Indian-melitensis—						
I	air/CO_2	*Nil*	+++	+++	strain rough, test not possible	..
II	air/CO_2	*Nil*	+++	+++	Do.	..
III	air/CO_2	*Nil*	+	+++	++1/320	++1/160

It will be observed that the first two strains were rough and, therefore, their antigenic structure could not be examined, but in all probability they are normal *melitensis* types. It is alleged that they were isolated some years ago from a cow and a goat in the Punjab. The third, was isolated from a naval rating in Poona hospital and, whilst growing in air and CO_2 and failing to produce H_2S, it grew but weakly on thionin and its antigenic structure appears to be more A than M. The origin of these *Melitensis* strains is obscure, but it seems possible that there is a slight occurrence of *Br. melitensis* infection in northern India.

TABLE XXVII

Ambiguous Indian Brucella *strains*

Strain's origin	CO_2 requirements at isolation	H_2S excretion	Growth on		Agglutination against	
			thionin	fuschin	mono-A serum	mono-M serum
Lucknow	CO_2	+++ ++ ++ +	+++	+++	1/160	1/5120
Jubbulpore	CO_2	+++ ++ + +	+++	+++	Negative	1/3560
Hissar	CO_2	+++ ++ + +	+++	+++	1/1280	1/80
Mukteswar	CO_2	+++ ++ + +	+++	+++	1/640	1/80
Mukteswar	CO_2/air	+++ ++ ++ ..	+++	++	1/320	1/80
Kanara District, Bombay (village)	CO_2	+++ ++ + +	+++	+++	1/160	Negative
Orissa (village)	..	++ +++ +++ +	+++	+++	1/320	1/80
Madras (village)	CO_2	+++ +++ ++ +	+++	+++	1/320	1/80
Bihar	CO_2/air	+++ +++ ++ ++ +	++	++	1/320	1/40

Pulses are used as an aid to glance estimation of degrees of reaction which are purely comparative. In the H_2S column the reaction has been registered for five successive days.

The 20 strains termed unclassified all differed from true *Br. abortus* types in one main characteristic *i.e.* they grew on both thionin and fuschin. Also the majority although undeniably *abortus* antigenically showed a slight tendency not to differentiate so sharply as pure *abortus* types on serological tests against monospecific sera and two were distinctly *melitensis* in this respect. One strain isolated from Patna and one from Mukteswar, whilst conforming to the above grouping, also grew in air at isolation.

Table XXVII illustrates the typing results of some of these ambiguous strains ; those not shown in the Table were unexceptional in their behaviour as A/M strains, and are typified by the Madras or Orissa village strains of the Table.

On first examining these strains, it was thought that a technical error had crept in, but, when the work was repeated keeping careful controls, it was found that if the dye concentration was low enough to permit the growth of *melitensis* on both media, and if ordinary European, and undoubted Indian, *abortus* strains were inhibited on thionin, but grew on fuschin, the results with these aberrent strains were always the same. Moreover, field strains were always typed in lots of 10 or 12 at the same time, and it is significant that, out of 49 strains, no less than 20 inescapably gave this ambiguous result. It is concluded therefore that this *abortus/melitensis* type is a common Indian *Brucella* variant, its constant characteristic being its ability to grow on both thionin and fuschin. Variably, it will grow in air on first isolation [2 cases out of 20 (10 per cent)] again its antigenic constitution shows in stability two strains out of 20 (10 per cent) being *melitensis* in antigenic form, the remainder conforming more or less to the *abortus* structure.

Regarding the origin of these aberrent types, it is significant that all the six strains isolated from the village endemic area of the Indian peninsula were of this nature. It is suggested, therefore, that this is a south Indian indigenous variant and the true *Br. abortus* type, hitherto found exclusively in organized farms, is an imported European strain. This finding strengthens the postulate that there is an area of indigenous infection almost exclusively confined to the Indian peninsula.

Further, a consideration of the part played by climate in *Brucella* dissemination suggests that, if there was a spread of infection from south India it would be likely to occur along the relative humid Himalayan foot-hills and perhaps descend into the Gangetic plain. It is of interest, therefore, that the *abortus/melitensis* strains, so far isolated from organized farms, have been found at Jubbulpore, not very distant from the endemic area of Nagpur and Raipur, and at Bihar, Lucknow, Mukteswar and Hissar, whither infection may well have spread in the manner suggested.

Agglutination absorption tests have been made on these *abortus/melitensis* strains to observe whether they contain any additional, hitherto undescribed antigen, but this does not appear to be the case.

Dissociation

Rough forms of *Brucella* can sometimes be recovered from the animate host, while they may also arise after prolonged artificial culture, especially if grown in fluids. The species *melitensis* dissociates very much more readily than the other members of the genus, and, amongst the present collection of Indian strains, two out of three *melitensis* types were rough when examined several sub-cultures after isolation, whereas, of 26 *Br. abortus* strains, one only was semi-rough at the culture of isolation. However, of 18 *Br. abortus* strains retested after some 15 or 20 transfers on liver agar, six had become partially rough and two wholly rough. None of the *abortus/melitensis* strains was rough at isolation, but of 13 retested after some 15 or 20 transfers four had become partially rough and one wholly rough. Rough strains are of course antigenically unstable and are not used for ordinary purposes.

Strains were examined for dissociation as follows. Two-day-old agar cultures were suspended in 12 per cent saline and adjusted to a density of 1 to 2 on the Brown's scale. The suspensions were placed in a boiling water-bath, readings for agglutination being made after 5, 15, 30 and 60 minutes. The suspensions were then removed and left on the bench until the following morning when they were again examined for agglutination. A strain showing any agglutination in these circumstances was regarded as antigenically unstable.

Antigenic sensitivity

The majority of the present collection of Indian strains have been tested for differences in antigenic sensitivity, and within the limits of ordinary error no differentiation has been observed—even the *abortus/melitensis* forms being unexceptional in this respect. Some of the results of this work are shown in Table (XXVIII).

TABLE XXVIII

The antigenic sensitivity of Indian Brucella *strains*

Strain No.	Type	Titre
F39	A/M	+1/1120
F40	A/M	++1/1280
F41A	A/M	+1/1280
F41B	A/M	+1/1120
F42	A/M	+1/1280
F43	A	+1/1120
F44	A/M	++1/1120
F45	A	++1/1120
F46	A/M	+++1/1120
F47	A/M	++1/1120
F48	A/M	++1/1280
Control (European)	A	++1/1280

A=*Br. abortus* A/M=*Br. abortus/melitensis*

Tests for antigenic sensitivity were carried out as follows. Standard antigens were prepared by suspending in 12 per cent saline 48-hour-old slant cultures of the strains to be examined, their density being very accurately adjusted to 1 on the Brown's scale. Stableforth's [1936] standard dried serum was reconstructed according to his instructions, but his final titration series was halved in saline. In a series of tubes, 0·5 cc. of the antigen to be examined was added to 0·5 cc. of each dilution of serum in the halved Stableforth series, thus forming a titration range of 1/640, 1/800, 1/960, 1/1120 and 1/1280 and the ultimate reaction after 24 hours incubation at 37°C was noted. In this range, type specific antigen prepared in a similar way had been previously found to react to about +1/1280, and this titre was accepted as the datum with which the end points of the field strains titrations were compared.

Virulence

As white mice are required in large numbers in assays of virulence, and, as these animals are difficult to obtain in India, only a small number of Indian *Brucella* strains could be examined for this property. The test is described by Priestly and McEwen [1938] and the results of the some estimations carried out in the present work are shown in Table (XXIX). For purposes of comparison tests on 3 foreign vaccine strains (V.1 V.4 and V.5) are included in this Table. These tests are mainly concerned with vaccine preparation and at present it is only desirable to note that they fail to kill white mice in considerably greater doses than do the field strains, whilst even among themselves their virulence is graded. Again, for control purposes, it was necessary to compare the virulence of Indian strains with that of type specific strains from abroad, and in so doing few mice were left for testing the local strains ; even then the interest centering round the A/M type led to four of these and only one *abortus* and one *melitensis* being examined. At this time, of course, it was not understood that the supply of white mice would fail entirely, due to war.

TABLE XXIX

The virulence of Indian field strains

Strain	Doses of bacteria					
	16×10^8	8×10^8	4×10^8	2×10^8	1×10^8	$0{\cdot}5\times10^8$
F.46 Indian CO_2 A/M	..	..	4	3	2	3
F.45 Indian CO_2 A	..	..	4	2	0	1
F.41 A Indian CO_2 A/M	..	..	5	5	2	2
F.40 Indian CO_2 A/M	..	..	5	3	0	1
F. 36 Indian CO_2 A/M	..	..	5	4	4	1
F.34 Indian M	..	..	5	5	4	4
A.1 European A	..	..	3	1	0	1
M. 1. Malta M.	..	..	5	3	1	1
V.1 [Cotton's 19]	5	4	1	..	..	..
V.4 [McEwens 45(6)]	2	1	1	..	..	..
V.5 [McEwens 45(20)]	5	5	2	..	..	..

Numbers = dead mice out of 5.
A = *Br. abortus* M = *Br. melitensis* A/M = *Br. abortus/melitensis* CO_2 = CO_2 *sensitive.*

The Indian strains that were typed were recently isolated and, except the *melitensis* variant, were CO_2 sensitive ; as might be expected the order of virulence proved to be as follows : *melitensis* most virulent ; *abortus/melitensis* of intermediate virulence and *abortus* least virulent. As repeated subculturing is supposed to lead to loss of virulence, a comparison with the overseas type-specific strains must be undertaken with caution, in as much as the Maltese strain has been isolated for six years and the English *abortus* strain must be many years old. If, however, due allowance is made for this, the experiment suggests there is little difference between Indian and overseas variants. In order to check this suggestion, in Table XXX the Indian strains are compared with an average result of four recently isolated English *Br. abortus* strains typed by Priestley [1938] and, if it is to be believed that tests done by different persons can be at all comparable, the foregoing conception of virulence seems to be substantiated.

TABLE XXX

Comparison of the virulence of Indian and European strains

Strains	Bacterial dose			
	$4\times10_2$	$2\times10_2$	$1\times10_2$	$0{\cdot}5\times10_2$
Indian M	5	5	4	4
Indian CO_2 A/M*	5	4	2	1
Indian CO_2 A	4	2	0	1
McEwens CO_2 A†	4	2	1	0

Numbers = dead mice out of 5.
*Average of 3 Indian village A/M strains.
†Average of 4 of McEwen's *abortus* strains (CO_2 sensitive and virulent) taken from J. Com. Path. 51, 284.
A = *Br. abortus* M = *Br. melitensis* A/M = *Br. abortus/melitensis* CO_2 = CO_2 sensitive.

Technical notes

For general information the following brief technical notes are appended.

Routine cultures : Unless otherwise specified all day-to-day cultures and the monthly sub-cultures of the type collection have been made on liver agar pH 6·6 to 6·8 [Huddleson 1939]. Up to the present, it has not been possible to preserve cultures by desiccation *in vacuo*, other than experimentally. However, with the recent installation of the necessary apparatus at the Imperial Veterinary Research Institute it is hoped that the type collection can be so disposed in future.

Isolation cultures : As long as tryptose has been available cultures intended for *Brucella* isolation have been made on tryptose (Difco)* agar or broth, (pH 6·9) otherwise liver media (pH 6·6 to 6·8) have been used.

Routine tests : Before use, stock *Brucella* strains have always been tested for roughness ; unless so specified, rough strains have not been used.

Dye media : Crystal-violet-liver (or tryptose) agar (crystal violet 1/700,000) has been used for primary isolation, when there has been risk of much contamination. In this and in dye media inhibition tests Grubler dyes have been used.

Technique of isolation : When several strains have been isolated from the same herd, subsequent, attempts have been made by cultural methods only. When no previous strains have been obtained, culture and guinea-pig inoculation have both been used. The actual field technique used in securing specimens has been described elsewhere [Polding 1943].

Section V. Diagnosis

Amongst the several recognized ways of diagnosing *Brucellosis*, by far the most popular is the blood serum agglutination test. The intradermal test is the only other method receiving practical attention and great claims are made for it in the U.S.S.R. ; but the double intradermal test entails the handling of animals thrice and a halt of four days to read a single test. The agglutination test, on the other hand, requires the handling of animals once and a halt of a few hours ; so that in the difficult touring conditions of India, and in dealing with the wild indigenous animal, the former method is much less practicable than the latter.

In the present work, therefore, the agglutination reaction has been exclusively used, and, in the interests of uniformity, an effort has been made to introduce a standard all India tube agglutination test.

The standard tube agglutination test

Experience in Europe has incontravertibly shown that it is often far from easy to standardize a biological reaction, even one so uncomplicated as a straight agglutination test. It has been shown for example that an antigen prepared at one institute may differ considerably from that made at another although the same technique and bacterial strains are used. The first step in standardization, therefore, is the exclusive universal use of a single antigen prepared as far as possible by the same staff and in the same laboratory. In consequence, a carefully prepared antigen has been offered to the provinces by the Indian Veterinary Research Institute at production cost only. Obviously, such an antigen should be used in the particular modification of the technique of agglutination for which it is designed, and the interpretation of the test's results should be in accordance with a predetermined schedule. A leaflet governing the use of the antigen is, therefore, obtainable from the Indian Veterinary Research Institute and has also been published [Polding 1943].

The tube agglutination test, as recommended by the Indian Veterinary Research Institute is believed to be the most critical and uniform that can be devised for routine work. Its use is urged, therefore, when an accurate diagnosis is required, that is to say, when a drastic decision, such as the disposal or slaughter of animals, depends on its result, or at other times when exact information is required.

* Baird & Tatlock Ltd : London.

The theory and practice of the standard agglutination test is as follows :

Antigen : Fundamentally, a *Brucella* antigen consists of a suspension of *Brucella* organisms in saline. It may therefore vary, (1) as to the character of the organisms used, (2) as to the density of the bacterial suspension, and (3) as to the concentration of NaCl in the vehicle. The character of the organisms employed is important in one outstanding respect ; the strain must be smooth, but contrary to the belief of many in this country, it is not particularly important wh ch variant is used, and some antigens are made from a combination of one, or more, strains of each of the variants *abortus* and *melitensis*, whilst others are prepared from a mixture of several strains of the same variant. In the present work, tests for variation in antigenic sensitivity have been made on numerous Indian and European strains (p. 146), and it has been found that, within the limits of experimental error, they all react to the same end-titre against a standard serum. Consequently, it does not appear to be very important from the point of view of origin, or type, which strain is selected, whilst the use of a multiplicity of strains complicates the work and leaves more loopholes for error. Actually, a single English *Br. abortus* strain is used for preparing standard *abortus* antigen and a single Maltese *melitensis* strain for making a *melitensis* antigen. The latter antigen has hitherto been offe.ed merely to satisfy an apparent demand from persons wishing to make comparative tests, but it serves no useful purpose for antigens of either variant will react to approximately the same titre with their heterologous or homologous antisera.

The density of an antigen is a matter of considerable importance, for variation of bacterial concentration will materially alter the end-point of a titration. In practice, antigens are encountered that vary from the transparency of water to nearly the turbidity of milk and while many complain of inconsistent results, there seems to be no satisfactory explanation why any individual adopts any particular density. The writer believes that a bacterial suspension should, in the first place, be sufficiently visible to make the detection of agglutination possible without the use of agglutinoscopes and for this, the final opacity, after dilution with serum, should not be much less than one half of the density of Brown's tube I. Secondly, within this limit, the density should be such as to be in nearly optimum proportion with the major antibody, when a serum is diluted to near its end-point. Some optimum proportion tests have been made to discover the antigenic density that gives an optimum reaction with serum, so diluted. In optimum proportion tests conducted near a serum's end-titre, the first appearance of agglutination is so trivial that the readings of different observers may disagree and the results of such tests must, therefore, be accepted cautiously. In each of the present tests, the selected dilution of serum was kept constant and the density of antigen varied. The systems were placed in a water-bath at 60°C. and observations were made at 5 minute intervals from the commencement of visible agglutination. The results of some of these tests are shown in Table (XXXI). The master titres of individual sera were obtained by testing them against antigen diluted to between tubes 1 and 2 of the Brown's scale.

TABLE XXXI

Optimum proportion tests of Brucella *antigens*

Time	Density of antigen*									
	5	>4	<4	3	2	<2	1	<1	$\frac{1}{2}$	<$\frac{1}{2}$
Serum (1) Stableforth's reconstructed serum, diluted 1/320 (titre +1/1280).										
11·15 A.M. . .	—	—	—	—	—	±	—	—	—	—
1·25 A.M. . .	—	—	—	—	—	+	+	—	—	—
11·22 A.M. . .	—	—	—	—	+	+	++	±	±	—

Optimum primary dilution of antigen = between 2 & 1 on Brown's scale.

TABLE XXXI—*contd.*

Time	Density of antigen*									
	5	>4	<4	3	2	<2	1	<1	½	<½
Serum (2) Stableforth's reconstructed serum, diluted 1/400 (titre +1/1280).										
11·15 A.M.	−	−	−	−	−	+	−	−	−	−
11·25 A.M.	−	−	−	−	−	+	±	±	−	−
11·35 A.M.	−	−	−	−	−	+	+	±	−	−
11·40 A.M.	−	−	−	−	−	+	+	±	±	−
Optimum primary dilution of antigen= between 2 & 1 on Brown's scale.										
Serum (3) Rabbit serum, diluted 1/640 (titre ++1/640).										
11·40 A.M.	−	−	−	−	−	−	±	±	−	−
11·55 A.M.	−	−	−	−	−	±	±	±	±	−
12·25 P.M.	−	−	−	−	−	±	±	±	±	±
12·50 P.M.	−	−	−	−	±	+	+	+	+	±
1·5 P.M.	−	−	−	±	+	+	+	+	+	±
After 20 hours bench temperature.	±	±	±	+	++	+++	+++	+++	+++	+++
Optimum primary dilution of antigen= less than 1 on Brown's scale.										
Serum (4) Same as serum (3), diluted 1/10 (titre ++1/640).										
11·15 A.M.	+	+	±	±	±	−	−	−	−	−
11·20 A.M.	++	++	+	+	±	±	−	−	−	−
11·25 A.M.	+++	++	++	+	+	+	±	±	−	−
Optimum primary dilution of antigen=5 or more on Brown's scale.										
Serum (5) Rabbit serum, diluted 1/2.5 (titre +1/10).										
10·50 A.M.	−	−	−	−	±	−	−	−	−	−
10·55 A.M.	−	−	−	±	+	−	−	−	−	−
11·0 A.M.	−	±	±	+	+	−	±	−	−	−
11·7 A.M.	−	±	+	+	+	±	±	−	−	−
11·45 A.M.	+	++	++	+++	+++	++	++	±	−	−
Optimum primary dilution of antigen= between 3 & 2 on Brown's scale.										
Serum (6) Same as serum (5), dilute 1/5 (titre +1/10).										
11·7 A.M.	−	−	−	−	−	−	±	±	−	−
11·15 A.M.	−	−	−	−	−	±	±	±	−	−
11·25 A.M.	−	−	−	−	±	+	+	+	−	−
11·35 A.M.	−	−	−	−	±	++	+	+	−	−
Optimum primary dilution of antigen= about 1 Brown's scale.										

* Density of antigen is indicated by the figures in the heads to the columns which correspond to the tube numbers of the Burroughs Wellcome Brown's scale. This density does not take into account the addition of serum, so in the final agglutinating system this density is halved.

In all, 21 optimum proportion tests were made and, on the whole, the results confirmed that, no matter whether a weak, medium, or strongly positive serum was used, if tests are made with dilutions approaching the serum's end titre, the optimum primary density of antigen is slightly less than 1 on the Brown's scale. When, however, serum is diluted to considerably less than its end-titre, the density of antigen that gives optimum reaction is greater, i.e., near about a final dilution of 2 on the Brown's scale.

Now, if it is agreed that an agglutination system should be most sensitive towards the end-point of a titration, these results show that antigen should be primarily diluted to about tube 1 on the Brown's scale. Its dilution when admixed with serum then becomes one half of tube 1 and it has been found that, when antigen is prepared to about this density, it agglutinates with Stableforth's reconstructed serum in a dilution of about ++1/1280. Stableforth [1936] himself prepares antigen at a density of 4 on the Brown's scale and his end-point with reconstructed serum is +1/480.

A third variable in antigen preparation is salt concentration. In the test being described, 12 per cent NaCl and 0·5 per cent phenol is used. A 12 per cent salt concentration is said to abolish zoning; it also ensures adequate self-mixing of the antigen and serum and does not appear to interfere with the test.

Antigens are prepared as follows: The selected strain is tested for S-R change and smooth strains only are used. After this test, the strain is sown on liver agar and incubated for 48 to 72 hours at 37°C. The growth is then washed off in carbol saline (12 per cent NaCl, 0·5 per cent phenol) and the bacterial density reduced to between 2 and 3 on the Brown's scale. This suspension is again tested for S-R change. A sample of the bulk is then diluted 19 parts to 1 and 18 parts to 2 and so on, in 12 per cent saline and each dilution is tested against reconstructed serum in the primary dilutions of 1/320, 1/400, 1/480, 1/560 and 1/640. The density of antigen that agglutinates nearest to +1/1280 is selected and the bulk antigen diluted accordingly.

Serum

The collection, preservation, and transportation of serum for the agglutination test have already been described [Polding 1943]. In selecting the serum dilutions for the standard test the series 1/10, 1/20 etc. has been chosen, rather than the other popular range of 1/25, 1/50 etc. The initial primary dilution (1/5) of the former is easier to prepare than that of the latter (1/12½). Further, in the former range there are four closely spaced dilutions (1/10, 1/20, 1/40, 1/80) in the critical portion of the range, whilst in the latter there are but 3 (1/25, 1/50, 1/100). For these reasons the former range is more practical.*

The quick agglutination test

Antigen for the quick agglutination test as recommended in India is prepared exactly in the manner of Huddleson [1939]. The actual technique and interpretation of the test is, however, a simplification of his method [Polding 1943].

In a country like India, the value of the quick test, as a supplement to the tube test, is unqualified. For, whilst its results are somewhat subordinate to the personal judgement of the operator and are therefore only a rough guide to the incidence of *Brucella* infection, the virtues of the test are manifold, e.g., (i) the abolition of the need for sending field specimens to the laboratory with all the attendant difficulties, and delays, (ii) the obtaining of a diagnosis on the day of the visit to the field outbreak, and (iii) the cheapness and compactness of the apparatus required. On the other hand, on account of its lesser accuracy, the quick agglutination test is only recommended for use in rough village survey work, for making preliminary tests before taking specimens for the isolation of *Brucella*, and for simple diagnosis, when all that is required is to establish the existence of Brucellosis in a herd. Moreover, this method can also provide valuable information in tests on fluids other than blood serum. For example, it is advantageous to quick-test the synovial fluid of bursal enlargements and the milk of aborting dams and only to attempt isolation of *Brucella* from these sources if their reaction is positive. Further, if time is available, it is advisable, in survey work, to quick-test the blood and milk of aborting animals and the blood serum and synovial fluid of animals with bursal enlargements

for, either of these fluids may be positive when the other is negative. A final important use of the quick-test is in vaccination work; here, it is usually desirable to vaccinate *Brucella* negative animals only and, if tests are made by the tube method, the animals must be twice-handled, once for bleeding and once several days later for vaccination. A better method is to secure the animal and take 5·0 cc. of blood into a bottle containing a few drops of a 20 per cent solution of sodium citrate. The mixture is shaken and the whole-blood tested immediately. The animals which have not yet been released, may now be vaccinated, or not, according to the result. Using whole-blood in place of serum, the quick-test is rather more difficult to read and, unless the operator has great experience, some sort of reading box, such as described by Huddleson [1939], should be employed. But failing this the plate should at least be screened from the direct rays of the sun, which, coming from above, obscure the reading, for it is light from beneath the plate that is required if the flocculation is to be detected amongst the turbid blood.

The accuracy of the agglutination test

Different authorities have placed the accuracy of the serum agglutination test, variously between 65 and 95 per cent, and it is fairly well established that perhaps one-third of all *Brucella* infected animals are transitory reactors, if indeed they react at all. In Indian herds, it has generally been found that about 70 per cent of animals, aborting during a true epidemic, are *Brucella* positive, whilst, in one very severe primary epidemic, 95 per cent was recorded. When, however, a minor epidemic is prolonged into an endemic, such a high ratio is not always seen, e.g., of 38 crossbred cows aborting during the period 1935 to 1940 and tested early in 1941, but 14 (36 per cent), were *Brucella* positive. In such cases, it is clear that very few temporary reactors have been picked out by the test and that the reactions of a number of permanent reactors has subsided. Nevertheless, this does not entirely account for the scarcity of reactors amongst aborters, for, of the above animals nine aborted during 1940 and of them, but two (22 per cent), were positive. It seems probable, therefore, that at the end of an endemic period, or perhaps in some secondary epidemics, the presence of permanent reactors is greatly diminished.

If whole herds, and not simply aborting animals, are considered, it appears that, when abortions are frequent, the incidence of reactors reflects to some extent the abortion rate, but when abortions are rare this relationship fails. In the early stages of sharp epidemics, when the abortion rate is in the order of 12 per cent to 15 per cent of the herd, the total percentage reactors in the herd will be from 45 to 60 and a number of animals are found to be infected that have not yet aborted. During a typical minor endemics, on the other hand, this rate is lower and was found, over an average of seven herds, to be 35 per cent. This last figure agrees exactly with that found by Doyle and Bechett [1936] in English cattle.

The diagnostic titre of the agglutination test has been much debated. In India, for example, it has been suggested that many indigenous cattle possess a week natural non-specific agglutination response to *Brucella* antigens, and that allowance should be made for this in diagnosis. But such a concept seems hardly tenable, for, in herds in which abortions do not occur, seldom a single animals can be found with even a doubtful reaction, whilst, in the comparatively disease-free districts of northwestern India, almost all the village animals that have been tested were strictly negative (p.117). On the other hand, in herds where abortions prevail, titres are usually quite high (1/320-1/2560) and sometimes very high (1/20,000), no matter the particular breed of cattle that is concerned. It is rarely difficult, therefore, to confirm an outbreak of Brucellosis by means of agglutination, but the diagnosis of infection in a single animal is only really informative when the test is positive. Operators should, therefore, view a single suspect animal in conjunction with the herd, as a whole, taking into consideration the magnitude and duration of an epidemic, the influence of intercurrent disease and all relevant data such as clinical symptoms, susceptibility, and epidemiology. To assist in interpreting the agglutination test, a list of commonly accepted diagnostic standards has been published [Stableforth 1936].

In buffaloes the position is entirely different, for, sometimes during primary epidemics of abortion in these animals, but 5-10 per cent dubious reactors can be found and it seems possible that some factor other than Brucellosis is at work. The aspect of specificity is discussed on (p. 188): here, it is important only to note that, if these primary epidemics amongst buffalo are indeed due to Brucellosis, then the agglutination test is wholly inadequate for their diagnosis.

Tests of buffaloes, co-habiting with endemically infected cows, and themselves aborting sporadically, show that 25 per cent of buffaloes as opposed to 38 per cent of cows are *Brucella* positive. In one flare-up of an endemic amongst co-habiting cows and buffaloes, 12 per cent of each population aborted within a few months and, whereas 70 per cent of aborting cows were strongly positive but 48 per cent of buffaloes were weak reactors, and some 30 per cent were doubtful.

In a herd of buffaloes suffering from a major *Brucella* epidemic (20 per cent abortions per annum), during the third year of the outbreak, at a time when some 60-70 animals had aborted, 25 per cent of the whole 215 animals were positive, while, three months later, a further 23 out of 152 previously negative animals had become so. In the fourth and fifth year of the endemic, about 50 per cent positive reactors were found. The highest positive incidence yet encountered in buffalo is 63 per cent of aborting animals in the fifth year of a fairly severe exacerbation of the disease. Finally, in the ordinary minor endemics of military farms, i.e., in cases where a trickle of abortion has been going on for years, the incidence of reactors is alike in cows and buffaloes (Table XXXII).

TABLE XXXII

The incidence of Brucella *reactors in aborting cows and buffaloes in minor endemics on military establishments*

—		Abortions	Positive	Doubtful	Negative
Cows	Actual	117	51	8	58
	Per cent	100	43	7	49
Buffaloe	Actual	67	28	5	34
	Per cent	100	42	7	50

15 farms observed.

The sum of the evidence, therefore, tends to show that once an outbreak of Brucellosis has become well established the *Brucella* agglutination test is about equally reliable in buffaloes and cows. On the other hand, in primary outbreaks of clinical abortion amongst cows, the incidence of *Brucella* reactors has up to the present been more than high enough to allow no doubt as to the specificity of the disease; but, in buffaloes, the incidence of reactors is either negligible, if the buffaloes are not in contact with cows, or if co-habiting, it is considerably less than in cows. This anomally can only be explained by supposing that either *Brucella* strains require adaptation to buffaloes, before they will provoke an agglutinin response, or, that certain primary epidemics of abortion in buffaloes are wholly or in part not due to *Brucella* infection.

SECTION VI. BRUCELLOSIS AND PUBLIC-HEALTH

In certain fairly well defined areas of the world, where the natural hosts the sheep and goat abound, *melitensis* fever is a serious disease of man. The main endemic region is the Mediterranean basin whence, it is supposed, infection has spread radially wherever communications are sufficient, and the environment is propitious. As will be shown later, it is also possible that infection can be carried by sea-routes, with man as the vector. An excellent example of the spread of this disease can be seen in its penetration into metropolitan France, where Brucellosis excited little interest until well after the turn of the century. In Sicily and southern Italy, on the other hand, it was described

as prevalent as early as 1872 and by 1938 Benzone [1936] claims that, next to Malta, Italy is the most infected country in the world. At this time, the fever had become common even in the extreme north-western province of Piedmont, adjacent to the French frontier. Again the disease had been recognized in the Iberian peninsula as far back as 1759. France, therefore, has been threatened along two landward approaches and it is significant that during the decade of the thirties, the two main endemic areas of that country were along the marches of the Pyrenees and a deep area lying *vis-a-vis* the Piedmont frontier. [Taylor, Lisbonne, Vidal and Hazeman 1938.] In 1938, these two endemic zones had not quite become confluent in the department of *Aude* on the gulf of Lyons. Moreover, the remoter sheep-populated departments of *Eure et Loire* and *Seine et Marne* and the western goat-populated department of *Deux et Sevres* have hitherto been disease-free. The infection has, however, spread along the Franco-Swiss frontier, or more probably through Switzerland itself, into the goat-breeding departments of *Meuse, Moselle, Rhin* and *Vosges* and so, into south-western Germany. Eyre [1936] defined the limit of its spread at the 46th parallel of latitude N., but by this date or a little later, the 50th parallel had almost been reached.

It has been suggested that infection was carried from the Mediterranean region to the new world *via* the milk goats of the conquistadores. On the Mediterranean littoral itself, other places known to be infected are Greece, Asia Minor, Algeria, Tunisia, Lybia, Palestine and Egypt, whilst further south some infection is probable in Transjordania, Erytrea, Tanganyika, Iran, Iraq, Aden, Somaliland and possibly the Cape (Fig. 13). *Melitensis* infection has also been described as far east as Singapore and Manchuria. It is evident, therefore, that in certain circumstances this disease can disseminate with ease, and it would be indeed extraordinary had not infection in some way penetrated into India.

From the year of Wright's and Semple's [1897] demonstration of the agglutination test for Brucellosis up to the time of the famous revelations of the Mediterranean Fever Commission [1905-07], report after report was published of undulant fever in this country. A crescendo was reached in 1907 and then very little more was heard of human infection in India.

It is remarkable that, with the crude facilities and technique of those times, officers were apparently able to isolate *Br. melitensis* with ease and certainty wherever they tried, viz., from 11 humans in Multan and Ferozepore [Lamb ; 1906] and from goats again in Ferozepore [Forster ; 1906] and that numerous positive and typical cases were described from all over India. Indeed, a perusal of the journals of that era must have forced contemporary readers to assume that *melitensis* fever was endemic in certain military cantonments with the inference that the Punjab, at least, was widely infested. Amongst the numerous places alleged to be infected were Ferozepore, Multan, Rawalpindi, Murree, Nowshera, Peshawar, and Mian Mir.

Furthermore, scrutiny of these records cannot fail to impress the critical reader with the fact that a gram-negative, non-CO_2 sensitive, cocco-baccillus, which was agglutinated by *Brucella* specific serum, was indeed being isolated from humans suffering from clinical undulant fever. Moreover, that Co_2 was not required to isolate these strains is reasonable proof that they were the variant *melitensis*. On the other hand, agglutination reactions were, on the whole, weak and a demonstration of infection in animals was infrequent and applied to a few cantonment-goats only.

From 1907 onwards, the spate of case reports suddenly subsides until at the present day it is difficult to obtain more than very rare reports of single cases of undulant fever in humans in India. But this surprising state of affairs can only be explained by one of three propositions, viz., (1) that the earlier reports were wholly incorrect (2) that in the period 1907-1939 human Brucellosis in India has practically died out, or (3) that undulant fever still occurs frequently and it is either not reported, or not diagnosed.

The earlier work must either be disbelieved as a whole, thus discrediting the work of several specialist officers, or accepted as a whole ; whilst at the present time, it is past belief that, frequent cases of so serious a disease as *melitensis* infection, could be missed in modern army hospitals. In the face of this, explanation must be sought in the rather improbable proposition 2.

Now it is queer, to say the least of it, that case reports in India subsided so soon after 1907, the very year succeeding that in which the infection rate in the British garrison at Malta was so greatly and dramatically reduced. But nearly all the cases of genuine Malta fever reported in India, occurred in cantonments and in the words of Scott [1939]. The disease was shown to be widespread in north-west India and *especially in places where British troops were stationed.* (The italics are the present writer's.) Can it be accepted then, that infection in Indian cantonments was brought by trooping movements from Malta, that dissemination from the human vector was purely localized, and, to round off the premise, that when fresh sources of infection from Malta failed, the local endemics, in the absence of a sufficient animal reservoir and possibly in the face of stricter boiling of milk, partially, or wholly died out ?

During the present investigation constant attempts have been made to stimulate medical interest in Brucellosis ; but it has been rightly contended that, during the wartime emergency, little effort could be spared for a disease of such apparent minor importance as Brucellosis. This branch of the work has, therefore, been considerably handicapped by the unavoidable circumstances of war.

Nevertheless, it is of deep epidemiological interest why a disease, so prevalent in man living on the goat populated Mediterranean littoral, should be apparently so rare in man in India ; where, in certain parts at least, sheep and goats are bred abundantly. At one time the simple explanation of the almost universal Indian custom of the boiling of milk was stressed as the explanation but, in France, Taylor, *et al* [1938] has clearly shown that amongst goat and sheep-breeders, at least, and possibly also amongst all who come in close contact with these animals such as stall-holders, housewives, and butchers probably 40-60 per cent of infection is transmitted to man, by contact. For a considerable section of the Indian public, therefore, the boiled-milk explanation fails and, if it can be assumed that Brucellosis in man would be diagnosed and reported were it a sufficiently prevalent-disease in this country, an assumption which is probably valid then it appears that, the reason for its rareness in man, must be its rareness in animals ; or, more remotely, that the conditions for contact-transmission prevailing in France are absent in India.

As for animals, occasional verified *Br. melitensis* strains seem to have been isolated in the Punjab and it is possible, therefore, that the disease has indeed reached this country probably *via* Persia or by sea from Malta. Nevertheless, it has been shown earlier in this paper that Brucellosis is rare in Indian sheep and goats, that indeed the only places where a regular, if low, incidence of reactors can be traced in these animals are in south India, where they are cohabiting with *abortus* infected indigenous cows. In the north-west, on the other hand, reactors are almost entirely absent.

The inference is, that, as in man, so in sheep and goats Brucellosis is uncommon in India. The whole position, therefore, appears to be one of disease transmission and the probable reason why *Br. melitensis* infection is rare, in man and animals alike, is because the main reservoir of near-Asia is shut off from India by vast expanses of sparsely populated sun-scorched desert and arid vegetationless hills. At the same time, even the trace infection that has so far reached the north of India is barely able to survive in the destructive climate of the Thar desert and valley of the Indus.

Nevertheless, looking back on the section on transmission it seems sufficiently clear that, if enough infection can traverse the Thar desert southwards then *Br. melitensis* infection might become an important endemic in south and central India.

A second means by which *melitensis* infection might reach south India is through the personnel of the fourth Indian division, which might have contracted sufficient undulant fever during its campaign in the Mediterranean to disseminate the disease after demobilization. In this respect, it must be recalled that man may eliminate *Brucella* organisms in the urine for months, or even years, after primary infection.

Br. melitensis is not, however, the sole *Brucella* variant pathogenic to man, and it seems possible that a variable number of Indians must become infected with *Br. abortus* by contact with infected animals. Serum-positive patients, suffering from fever, are encountered from time

to time, notably in the North West Frontier Province and the Punjab, but, until the responsible strain is isolated, it will not be known whether these fevers are *melitensis* or *abortus* in type.

In the endemic areas of south India, the behaviour of the indigenous A/M strain in man would be of particular interest. During the last several years six or seven cases of undulant fever have been described at the King George V hospital, Vizagapatam [Pandalai and Raman 1941]. Regrettably such strains as were isolated from these cases were discarded untyped, but, in the writer's opinion they were probably A/M in character.

In view of the practice of boiling milk and the peculiar climatic conditions of south India, it would also have been of great interest to have discovered the incidence of Brucellosis in man in the village endemic areas of the peninsula. In consequence, a list of infected districts (Table XXXIII) was given to the medical authorities, who, in their turn, addressed a questionnaire to the local doctors concerned. From all districts the reply was that no undulating-type of fever was encountered in humans; but that, in future, undiagnozed cases of pyrexia would be tested for *Brucellosis*.

TABLE XXXIII

List of districts in which Brucellosis is endemic in cattle and in which undulant fever in man is not reported

District	Province
Nellore	Madras
Madura	Madras
Kollegal	Madras
Sambalpur	Orissa
Nawapara	Orissa

These replies are about the some of the information that can be obtained at present and, on the whole, it appears that, if the disease occurs at all, and it would be surprising if it did not, then it does so within insufficient severity or frequency to make itself noticeable to the local medical authorities. When the disease is due to *abortus* strains, symptoms are often so slight, that in view of the numerous transient undiagnozed fevers, which are either attributed to malaria, or treated empirically and are always regarded as commonplace, it would not be surprising if most cases were overlooked. Undulant fever due to *melitensis* infection, on the other hand, is usually severe and it is hardly possible that in army circles, at any rate, it would pass undiagnozed. It must be conceded, therefore, that *melitensis* infection of man must be relatively rare in India, but that an unknown amount of infection with the *abortus* or *abortus/melitensis* strains may pass unnoticed in south India. This view is supported by the admission in reply to the above-mentioned questionnaire that undiagnozed fevers do exist in *Brucella* endemic tracts of south India.

In view of what has just been written concerning the climatic barrier of Persia and near-Asia to the eastward migration of *melitensis* into India, the question might aptly be asked, why this infection continues to be so prevalent on the arid and sun drenched Mediterranean seaboard?

As far as man is concerned the custom of drinking unboiled milk is, to the writer's knowledge, universal amongst the uneducated Maltese, and this probably holds good throughout most of the northern littoral. Infection in the goat, therefore, ensures infection in man. As for the goat, this animal breeds seasonally kidding as a rule in February, March and April and although the Mediterranean is generally supposed to be arid, this season, at least, is one of extreme cold and violent rainstorms.

SECTION VII—THE CONTROL OF BRUCELLOSIS IN INDIA

Control by hygiene

For very many years, the usual systems of controlling Brucellosis have been based on the serum agglutination test; whole herds were tested at short intervals, and reacting animals discarded. But, because of the defects of the test (p. 152), control on such a basis, whilst often resulting in a reduction of the number of abortions, only succeeded in eradicating the disease entirely, when the tests were very frequent and the system very conscientiously enforced. Furthermore, the question of the disposal of reactors was a matter of considerable difficulty. Some adopted the policy of selling to the butchers, a procedure often entailing the sacrifice of very valuable animals. Others merely sold on the open market, which resulted in the spread of Brucellosis, whilst a third school attempted whole-time segregation. This last system, though possibly the most praiseworthy morally, was, in practice, uneconomical, for it not only demanded separate farm buildings, separate labour and separate feeding-arrangements, but, quite often, separate herds were required for animals suffering from other diseases as well. The writer has seen on one farm a disease-free herd, a Brucellosis herd, a Tuberculosis herd, a Johne's disease herd and even herds suffering from combinations of these diseases, manifestly an absurd situation.

In India, the test and slaughter system for cattle is almost ruled out because of religious sentiment and the poorness of the beef market; but it has occasionally been used with success in goat-herds, wherever the sale of infected animals to the butcher was possible and the drain of infected animals was slight. The test and sale method is, in India, as elsewhere epidemiologically bad practice. There remains, therefore, the system of test and segregation, and, as in India labour is cheap, extensive grazing is often available, and the climate permits the simplest of housing for animals, the method is probably more feasible here, than elsewhere. It is, nevertheless, irksome and in view of the slight degree of infection often encountered in indigenous herds, the high natural resistance of the zebu and the sterilizing power of Indian sunshine, a modified form of segregation has been evolved for use in this country. Furthermore, as routine half-yearly, let alone two-monthly, tests are rarely possible, testing is largely disregarded and the system centres round hygiene and segregation, during the dangerous periods.

The system of hygienic segregation has already been described [Polding 1943]; but, as some modifications have since been introduced, a short restatement seems necessary here. Firstly, it has clearly to be understood that the method can be most suitably applied in the following special, but frequently encountered, circumstances: (1) when the frequency of abortion is less than 5 per cent of the breeding herd *per annum*; (2) when the disposition of the farm lends itself to cleanliness; (3) when frequent purchases of in-calf animals from endemic centres are not contemplated; (4) when for the greater part of the day the stock can be dispersed for grazing over a wide area and (5) when climatic conditions are good. These are the ideal circumstances; but they apply to many farms, such as the Punjab Grantee Estates and private and government farms situated in north and central India. Moreover, it is probable that, provided extra care be taken, the system would be serviceable in almost any clean grazing farm not subject to a major epidemic or abnormal cattle movements. Contra-indications are: (i) when a major epidemic has taken a good hold on the farm; (ii) where the monsoon is prolonged and severe; (iii) where animals are confined to dirty and crowded compounds; (iv) when cattle purchases from Bombay and Calcutta, or other endemic areas are unavoidable.

The first step in the procedure is the provision of segregation quarters, and where the climate permits, it is better to dispense with any form of building whatever. Depending on the size of the herd, one or more fenced paddocks should be made, well away from the main farm buildings, but convenient of access (say some 2 to 3 hundred yards from the cattle yards). Each paddock may contain a simple thatched roof on poles for shelter and should preferably be enclosed by a double fence with 2 yards of neutral ground between. A paddock should be on a site which will not become waterlogged. When cold or excessive rainfall prohibits the use of paddocks, one or more simple calving boxes should be erected on sites similar to those chosen for paddocks. Boxes should consist

of a drained cement floor surrounded by six-foot brick walls, cemented on the inside to the top. A simple roof, carried on poles projecting four or five feet above the walls and with overhanging eves, completes the structure. The entrance does not require a door and may be closed by bars fitting in slots, whilst drainage should be carried onto cultivated land so as to be inaccessible to cattle. The size and number of boxes or paddocks are matters for some deliberation; if it is uncommon for more than one or two recently aborting animals to be on the farm at one time, then a single box or paddock with accommodation for two will be sufficient, but, as it is not desirable to place more than two or at the most three recently aborting animals in the same paddock or box, when abortions are more frequent, proportional accommodation must be arranged. In fixing this, it must be borne in mind that the longest continuous period a single animal will be in isolation is 60 days.

The periods during which *Brucella* infected animals should be isolated in these premises are as follows:

(1) From the first signs of abortion or premature delivery until 60 days after the act of delivery.

(2) All cows isolated under (i) from about three weeks before the anticipated date of the subsequent parturition, or from the time of second abortion or premature delivery should it occur. (*N.B.*—animals should be watched for impending abortion in this pregnancy) until 30 days after delivery.

(3) All cows isolated under (1) and (2), from about three weeks before the anticipated date of the next parturition, until cleansing and vaginal discharges have ceased after delivery.

Where facilities are available and where the adoption of extra special precautions seem necessary to check a moderate epidemic in a valuable herd, the following additional refinement may be adopted. The veterinary officer dealing with the farm should obtain, on loan, a 'quick-test' apparatus and, once every second month, a day should be set aside for testing all previously healthy cows that are pregnant between the beginning of the fourth and the end of the fifth month of gestation. Positive reactors should be grouped into a segregated herd, living in temporary quarters and grazing separately. As each positive animal either aborts or reaches three weeks of term, it should be isolated in the same way as aborting cows in (1) above. As each healthy cow calves, it should be tested and if positive segregated as before. This additional refinement should not be necessary for longer than a year or two and can be stopped when a material drop in abortion rate is noted.

Hygienic precautions should be taken in all cases of abortion [Polding 1943].

A system, similar to the one just described, has been in force in all military farms, since early in 1941, but the enormous expansion in cattle concentrations and movements due to war, entirely masked any benefits the procedure may have conferred. However, general indications from farms unaffected by the war and practising some similar form of hygiene, all tend to show that Brucellosis can be checked by this means.

The theory of vaccination

The present popular form of vaccination against Brucellosis has been so discussed in current literature that much introduction to the subject is unnecessary. In America, Cotton, Buck and Smith [1934] showed that prolonged artificial culture of *Brucella* strains reduced their pathogenicity for animals and they secured such a strain of lowered virulence, identifying it as strain No. 19. They further showed that the injection of this strain failed to produce visible lesions in guinea-pigs or generalized infection in cattle, although it seemed to invoke an immunity against reinfection with field strains. Unhappily, the injection of Cotton's 19 also produced a serum response, which, if present in adults, would confuse to a point of break-down American's interestate system of control by test and disposal; a system which the authorities were loathe to discard. It was found, however, that, if 4 to 8 months-old calves were injected, the resulting serum reaction had disappeared by adolescence, and it seems possible that Americans were prejudiced in favour of calfhood vaccination by their desire to retain the interestate testing system while Cotton's vaccine was on trial. The principle of calfhood

vaccination can be defended on the ground of safety, for, the longer the period between vaccination and parturition the less the chance of bacterial dissemination at calving. But although the Americans suggest that the optimum age for immunization is 4 to 8 months, many believe that they have not proved their case [McEwen 1941]. It is well known, for example, that very young calves are naturally strongly immune and that this immunity is entirely lost by puberty, and without experimental proof it is difficult to believe that a weak immunity, conferred by a low-virulent strain, at 4 to 8 months of age will survive into the first or second calving; it is nonetheless possible, that by vaccinating between 4 to 8 months, i.e., when the natural immunity of calfhood is beginning to fade, the animals are caught at the most suitable moment for re-immunization.

A very large scale trial of this system is at present being made in United States. On the whole the results of first calvings seemed promising, but at the second calving they were less so. In this connection it has been rightly pointed out that, as heifers are usually kept separate from the adult herd during their first pregnancy, they are much less exposed to infection than second calvers who spend a whole pregnancy in contact with infected adults.

In England, McEwen has worked in great detail along parallel lines, and, by artificial passage has produced from an almost a virulent parent strain (No. 45), two sub-strains [No. 45 (6) and No. 45(20)] each of rather greater virulence than its predecessor [McEwen 1940]. He has also developed one or two systems of measuring virulence (p. 146) and has further shown that a strain's immunizing value is proportional to its virulence. With reason, therefore, he suggests that a vaccine strain should be of the greatest possible degree of virulence, short of its being virulent enough to cause generalized infection in cattle; he adds that to achieve and maintain this degree, in practice, is not at all easy; McEwen favours the vaccination of heifers and his strain No. 45(20), being rough, does not invoke an agglutination response in the recipient, which is an advantage, as confusion over blood tests need not arise.

Very many trials have been made of vaccines prepared from either Cotton's or McEwen's strains, and, whilst the results have not been uniformly favourable, there is an overall indication that some measure of protection can be obtained from their use.

THE CHARACTER OF THE VACCINES USED IN INDIA

Cotton's strain No. 19, McEwen's Nos. 45, 45(6) and 45(20) together with another similar strain Huddleson's No. 805 have been brought to this country and have been tested for virulence under Indian conditions.

As Cotton's and Huddleson's strains were brought out at the commencement of the scheme, they have been tested more thoroughly than McEwen's which were received much later. Owing to a shortage of funds and accommodation, it was not possible to conduct controlled tests on these strains in adult buffaloes, zebu and crossbreds as was manifestly desirable. Experiments have been made, therefore, in crossbred calves, goats, guinea-pigs and white mice.

Examination of vaccine strains

Test I. Cotton's strain No. 19 and Huddleson's 805, having been in the writer's possession for a very long period, were examined as to their general characteristics. They were found to be typical *Br. abortus*, to be smooth and of standard antigenic sensitivity.

Test II. Strains 19 and 805 were examined for their virulence in guinea-pigs. The guinea-pigs received 3500×10^6 organisms in 1·0 c.c. of saline in the manner shown in Table XXXIV where the animals' subsequent serum responses are also recorded.

The guinea-pigs were killed on the 35th day and the liver, spleen and kidneys of each animal were plate-cultured. *Brucella* organisms did not appear on any of these cultures.

Test III. The pathogenicity of Cotton's No. 19, Huddleson's 805 and a recently-isolated, CO_2 sensitive, Indian-field-strain, out of a buffalo, was compared in goats. The virulent control

strain was of the *abortus* type and smooth, while the second sub-culture from isolation was used. In each case the dose administered was 4000×10^6 organisms in 1·0 c.c. of saline, given subcutaneously. The recipients were grouped as shown in Table XXXV.

TABLE XXXIV

Result tests I and II

Strain No.	Route of injection	Guinea-pig No.	Serum response at intervals after injection		
			10 days	28 days	35 days
Cottons 19	I/P	95	+1/1280	++1/640	+++1/320
	S/C	97	+++1/320	++1/320	+1/160
Huddleson 805	I/P	106	+++1/640	++1/40	+++1/40
	S/C	115	+1/320	+++1/80	+1/80

TABLE XXXV

Grouping of goats in test III

Strain injected	Goat No.	Sexual phase	Age of goat in months
Indian field strain, group F-6	72	non-pregnant	12
	162	pregnant	36
	26	pregnan	60
Cotton's No. 19, group A-4	188	pregnant	14
	493	pregnant	36
	119	pregnant	48
Huddleson's No. 805, group A-5	64	pregnant	12
	21	pregnant	24
	431	pregnant	60

The mean serum response of the three animals in each group was estimated from a series of tests with the results shown in Table XXXVI.

TABLE XXXVI

The serum response of goats in test III

		4	6	9	11	18	30	45	70
Field strain	*Nil*	+1/160	+1/320	++1/2560	+++1/2560	+1/1280	++1/640	+++1/640	+++1/640
Cotton 19	*Nil*	+1/160	+1/320	+1/320	+1/2560	+1/640	+1/320	++1/320	+1/80
Huddleson	*Nil*	+++1/40	+1/160	+1/640	+1/320	+1/80	+1/40	+1/40	+++1/10

The response at the field-strain was normal and persistent; that to Cotton's 19 more transient and that to Huddleson's 805 quite transient. The blood of these goats was cultured at various intervals (Table XXXVII) and it was found that the virulent strain produced a bacteraemia in all three of its recipients, whereas no bacteraemia was demonstrable in members of either group receiving the vaccine strain.

TABLE XXXVII

Haemoculture of goats of test III

Goat No.		6			9			11			15			19		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
72	F.6	—	+	+	C	C	C	+	+	C	+	+	+	—	+	+
162	F.6	—	—	—	C	C	C	+	C	—	—	+	+	—	—	—
26	F.6	—	—	—	—	—	+	+	+	—	+	+	C	+	Tube broken	
188	A.4	—	—	—	—	—	—	C	C	C	—	—	C	—	—	—
433	A.4	C	C	C	C	—	C	—	—	—	—	—	C	—	C	—
119	A.4	—	C	C	—	—	C	—	—	—	—	—	—	—	—	C
64	A.5	—	—	—	C	—	—	—	—	—	—	—	—	C	—	C
21	A.5	C	C	C	C	—	—	—	—	—	—	—	—	—	C	—
431	A.5	C	C	C	—	—	—	C	C	C	—	—	—	—	—	—

+ = *Brucella* isolated from plate. — = Sterile plate.
C = Contaminated plate.
The blood culture from each date was plated thrice at the end of the first, second and third weeks of incubation. Sub-heads 1, 2 and 3 refer to these plates.

The birth secretions of all animals were cultured (goat No. 72 became pregnant during the experiment) and abortions were recorded (Table XXXVIII). The results showed that the virulent strain caused abortion in two out of three goats and general genital infection in all. All the recipients of the vaccine strains, on the other hand, carried their kids to full-term and appeared to be free from genital infection.

Test IV. The pathogenicity of Cotton's strain 19 was compared with that of Indian-field-strains in guinea-pigs. Saline suspensions of Cotton's 19 and Indian-field-strain F. 33 were made in concentrations of 10,000, 1,000 and 100 million organisms per 1·0 c.c. Pairs of guinea-pigs were subcutaneously vaccinated with 1·0 c.c. of these doses. Eighteen days after these inoculations, blood cultures were prepared from all animals; all cultures from the guinea-pigs receiving the vaccine strain irrespective of dosage were sterile, but *Brucella* organisms were recovered from one guinea-pig, receiving 10,000 million, and from another receiving 100 million virulent organisms. The virulent strain was, therefore, capable of producing a bacteraemia on the 18th day even in the smallest dose, while the vaccine strain did not. One guinea-pig receiving 100,000 million Cotton's 19 organisms died during the procedure of blood collection, and plate cultures of its liver and spleen produced one, and five, *Brucella* colonies respectively on each culture—a culture from its kidney was sterile. It appears, therefore, that some generalized infection must have been caused by Cotton's 19 at the 18th day. The remaining animals were killed on the 31st day after injection and their livers, spleens and kidneys plated. No *Brucella* colonies were found on the plates from the animals receiving Cotton's strain, which had evidently thrown off any infection that might have been present on the 18th day, whilst, with the exception of one animal (the kidney of which was negative), all recipients of the virulents strain gave numerous colonies from all sources of seeding.

Test V. The effect of varying doses of Cotton's strain 19 was tested in cross bred calves, aged 4-8 months. Inoculations were given subcutaneously to pairs of calves, in doses of 800, 4,000, 8,000 and 40,000 million organisms, in saline. The resultant temperature reactions appeared to be unaffected by the magnitude of the dose, all of them reaching a peak of about 104° to 105° F on the second day, after which, they fell rapidly to normal. A hard and fairly painful swelling reaching a maximum on the third day and disappearing in about a week developed at the site of injection. The animals continued to feed and appeared otherwise unaffected by the injection. All calves responded with a high serum reaction which peaked about the 12th day with titres in the order of +/—1/2560. Blood cultures prepared on the 9th, 12th, 15th, 18th and 22nd day after injection were all sterile. The gradation of doses had no obvious effect on any of their reactions.

TABLE XXXVIII

The results of parturition of the goat of test III

Goat No.	Date of kidding and remarks	Results of culture of			
		Placenta	Foetus	Milk	Vaginal swabs
Group F.6—					
72 . .	Aborted one male kid, 20th December 1940 . . .	+	+	—	+
162 . .	Kidded one full term live kid, 25th June 1940 .	+	0	—	+
26 . .	Kidded two dead premature kids, 27th June 1940 .	+	+	0	+
Group A.4—					
188 . .	Kidded one healthy kid, 4th October 1940 . . .	Lost	0	—	—
433 . .	Kidded one healthy kid, 4th October 1940 . . .	—	0	—	—
119 . .	Kidded two full term live kids, 27th June 1940 . .	—	0	—	—
Group A.5 —					
64 . .	Kidded one healthy kid, 30th October 1940 . . .	—	0	—	—
21 . .	Kidded one healthy kid, 15th August 1940 . . .	—	0	—	—
431 . .	Kidded one healthy kid, 6th October 1940 . . .	—	0	—	—

+ = *Brucella* isolated ; — = Brucella not isolated : 0 = culture. Negative plates were made on six occasions, on milk and swab-culture.

Test VI. The avirulence of vaccine strains Cotton's 19 and McEwen's 45(6) and 45(20) was compared with each other and with a selection of virulent strains, by means of toxicity tests in white-mice. The result of this work appears in Table XXIX, p. 147. In another experiment of the same kind Huddleson's 805 was compared with Cotton's 19. According to the criterion of these tests the descending order of virulence of all strains examined appears to be (1) all field strains, (2) McEwen's 45(20), (3) Cotton's 19 (4), McEwen's 45(6) and (5), Huddleson's 805 and such information as can be obtained from the literature confirms these findings.

The criteria of these tests have differed, but all results show that the vaccine strains are, to a varying degree, less virulent than Indian-field or type-specific strains, while Test III clearly illustrates the inability of strains 19 and 805 to produce generalization or abortion, at least in goats. Indeed, the sum of the experimental evidence suggests that all five strains are safe to use and that

the relative virulence of the three principal ones is fairly accurately portrayed in Table XXIX. If then the immunizing value of a strain is indeed proportional to its virulence, and, if a single strain only is to be used, plainly either McEwen's No. 45(20), or Cotton's No. 19, should be selected as being of the highest virulence consistant with safety. If however, the use of a combination of strains or even two injections is contemplated, then the choice becomes more difficult and trials would have to decide, whether to give a strain of low virulence followed by one of greater or whether some other combination was preferable. There is an enormous field of research in which to decide (i) the optimum age, or sexual phase, at which to inject cattle with—(a) a single dose, or (b) 2 doses; (ii) this optimum size of any one dose; (iii) whether one, or more, strains should be used and if so which; (iv) a means, acceptable to all, of standardizing the degree of virulence of a vaccine strain and (v) whether vaccination should be repeated in later life and, if so, when and how often. Again, there is much work to be done, in assessing the correct vehicle for the vaccine. It has been shown for instance that certain microbes survive longer in some fluids than in others and it is well known that some bacteria die rapidly in a simple saline suspension. These points are of great importance in India, where periods of transport extend into days and variations in climate are extreme. Consequently, as the remains of the vaccine, hitherto returned from field during the present scheme, have been invariably found to be dead, a series of experiments was devised to study the survival of *Brucella* in fluids. The enumeration of the bacteria surviving in suspensions entails the use of serial dilutions and plate-counts—a method which is subject to many small personal differences in technique. Except in the preliminary trials (p. 163) where changes of methods were tried, the standard technique adopted in the work to be described was as follows. With a clean sterile 1·0 c.c. pipette, 1·0 c.c. of the bacterial suspension was transferred to exactly 9·0 c.c. of diluent in tube 1 of a dilation series and mixed by blowing through the pipette, whilst moving its tip up and down the column of fluid thrice. With a fresh similar pipette 1·0 c.c. of the mixture in tube 1 was transferred to 9·0 c.c. of diluent in tube 2 and this process of transferring and mixing of fluids and discarding of pipettes was continued until the requisite number of dilutions were prepared. Although different vehicles were used in the preparation of various suspensions, unless otherwise stated, the diluting fluid used throughout was 0·85 per cent NaCl in distilled water. The *p*H of this fluid was not adjusted, but it was generally slightly acid—(*p*H 6·0 to 7·0)—a reaction which subsequent experiments showed to be favourable to *Brucella*. Huddleson [1943] stipulates that, in making plate-counts of *Brucella*, the diluting fluid should contain 0·1 per cent of tryptose, as this makes the counts more regular. Such an addition was not used in the present work, as, on the whole, the count results were satisfactory and the use of tryptose entailed the introduction of another unknown.

Plates were prepared from the dilutions by pipetting with a fresh 1·0 c.c. pipette, 1·0 c.c. from the selected dilution tubes in ascending order of bacterial density, without changing the pipette. Then the melted media—about 12 c.c.—was poured at a temperature of 45° to 48°C. and the plate's contents mixed by the usual rotating and reciprocating movements. Plates were in 4 in. in diameter.

It is perhaps unnecessary to give full details of the earlier series of experiments (Tables XXXIX to XLII) but their findings may be summarized as follows. (1) The death-rate of *Brucella* in a saline vehicle is probably proportional to the temperature of storage (Table XXXIX). (2) The death-rate of *Brucella* in a saline vehicle, stored at 26°C. is regular and relatively slow (Table XL). (3) Conversely, the death rate of *Brucella* in a saline vehicle, stored at 42°C. is relatively rapid for the first day or two and thereafter less rapid, while in this environment the death-rate is little affected by the addition of small concentrations of gelatine or tryptose (Table XLI). (4) *Brucella* stored at 42°C. in a saline vehicle probably increase in viable numbers for upwards of 12 hours, but before the twenty fourth hour is reached a very rapid death-rate supervenes and the viable count decreases speedily (Table XLII). These preliminaries suggested that the period of storage during which the effect of different environments could best be studied would coincide with the phase of rapid death, say from 18 to upwards of 48 hours of storage. Moreover, as limited facilities prevented the use of more than three variables in each test, a standard storage temperature—considered to be an average adverse plains temperature—was adopted in most tests, viz., 42°C. (103°—5F°).

TABLE XXXIX

The survival of Brucella *in saline suspension at various temperatures*

Time in days	Storage at 0—5°C.	Storage at 20—25°C.	Storage at 37°C.
5	16,000	3,400	2,000
9	16,000	5,000	1,500
12	..	..	500
18	650	192	40

Figures = Count in millions per c.c.
Technique = Standard count on tryptose agar.
Culture = Cotton's 19, 48 hrs. old.
Vehicle = Saline 0·85 per cent *p*H unknown.
Density = Brown's scale 9-10.

TABLE XL

The survival of Brucella *in saline suspension after transit*

Time in days	Stored at Mukteswar at 70°F.	Sent to Lahore and stored at room temperature average=71°F. then returned	Sent to Lahore and stored at pit temperature average = 77°F., then returned
0	6700	6700	6700
10	2640	..	..
15	1180	2·5	82
19	1080	2·5	2·6
23	240	Contaminated	2·6

Figures = *Brucella* count in millions per c.c.
Bacterial suspension = Cotton's 19, 48 hours old growth in 0·85 per cent saline at a density of Brown's scale 5-6.

TABLE XLI

The survival of Brucella *in various fluid vehicles*

Time in days	Saline	Saline +2·5 per cent gelatine	Saline +0·5 per cent glycerine	Saline +0·1 per cent tryptose
0	8500	7800	8500	8700
5	10·5	18·0	15·0	18·0
19	10·8	11·3	Contaminated	10·2
25	14·8	8·5	..	3·6
40	3·6	4·0	..	5·6

Figures = *Brucella* count, in millions per c.c.
Culture = Cottons's 19, 48 hours incumbation.
Vehicles = as shown in table.
Brown's scale = 5-6.
Technique = Standard count.
Medium = Tryptose.
Storage = 42°C.

TABLE XLII.

Short term count of the survival of Brucella *in saline vehicle*

Time in hours	Count in million per c.c.
0	2900
4	4400
8	4500
12	5400
24	1650
51½	104
65	58

Culture = Cotton's 19, 48 hours old.
Browne' scale = 5·6.
Medium = Tryptose.
Vehicle = 0·85 per cent saline.
Technique = Standard.
Storage = 42°C.

The effect of alterations in the *p*H of the vehicle was first studied and in two experiments [Experiments I and II, Figs. 4 and 5] it was shown that *Brucella* vaccines suspended in 0·85 per cent NaCl, buffered with M/50 Sorensen's phosphates, and stored at 42°C., tolerated the acid range *p*H 6·0 to 6·8— but were intolerant of the extremes—*p*H 5·4 or *p*H 8·0. Moreover, during both experiment it was found that, although buffered, the reactions of the substrates drifted towards alkalinity. This was first observed in Experiment I and it was thought to be due, perhaps, to an adulteration of the suspension with substances derived from the agar. In Experiment II, therefore, this possibility was avoided by washing the bacteria in a single change of vehicle, but although minimized the tendency was again noted. To lessen the effect of this drift, an optimum *p*H of about 6·4 to 6·6 was selected and used in all experiments subsequent to Experiment III.

In Experiment III, Fig. 6 the effect of the presence of inorganic salts was examined and it appeared that survival in buffered distilled water was not so good as in buffered physiological saline while a complex salt solution was no improvement on the sample.

Experiment IV was designed to show the effect of the addition of small quantities tryptose, gelatin or glycerine to the optimum buffer evolved in the foregoing tests. The records, Fig. 7 suggest that, not only did the most regular death-rate occur in the tryptose solution, but here also the total survival was to a small degree the best, gelatin proving a good second.

The close of the scheme curtailed any further attempt to improve the keeping qualities of the vaccine, and so the survival rate in the ' improved ' vehicle was compared with that in common saline. The storage temperatures selected were 42°, 30° and 0·0C. and the solutions were counted over a period of a few weeks. The respective merits of the optimum and saline substrates may be judged from an inspection of Fig. 8 and four major points seem to emerge from this work viz., (1) during five or six weeks storage at 0°C., the death-rate in either vehicle is trivial. (2) at Indian plains temperatures (*i.e.*, 70 to 110°F.) the viable count falls rapidily in relation to the time and temperature of storage. (3) In moderate temperatures at least, it seems probable that an initial phase of mortality is followed, in three or four weeks, by one of multiplication. (4) At the lower temperatures, the optimum vehicle gains some initial advantage over the common saline, a lead which is held but not increased, thereafter. At 42°C. the optimum vehicle appears to have a decided advantage over the saline.

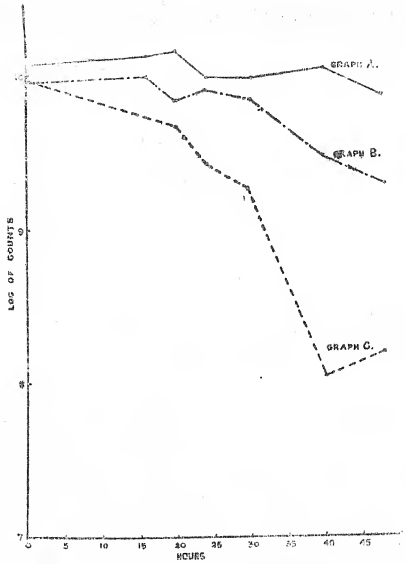

FIG. 4. *The effect of *p*H of the vehicle on the keeping qualities of *Brucella* vaccines.

A=*p*H 6·0
B=*p*H 7·0
C=*p*H 8·0

Conditions of experiment in fig. 4.

Vehicle. Glass distilled water containing NaCl (pure) 0·85 per cent and buffered with Sorensens phosphates M/50 to the *p*H shown.

Storage. The temperature of storage was 42° C.

Suspension. The suspension was made from a 48 hours old, culture of Cotton's strain 19, its density was 5 on the Browns scale.

Table of bacterial counts in hundreds of millions per c.c.

Hours	*p*H 6·0	*p*H 7·0	*p*H 8·0
0	117	90	93
16	130	100	64
20	140	69	46
24	98	79	27
30	95	67	13
40	109	29	1·1
48	70	19	1·5

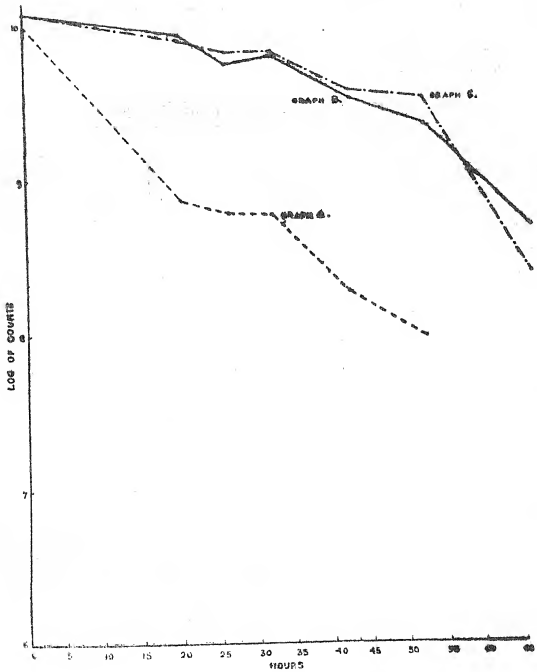

FIG. 5. *The effect of *p*H continued.

A=*p*H 5·4
B=*p*H 6·0
C=*p*H 6·8

* For conditions and table of counts see overleaf.

Conditions of experiment in fig. 5.

Vehicle. As for fig. 4.

Storage. As for fig. 4.

Suspension. As for fig. 4, but the suspension was washed before use by centrifugation and resuspension in the same vehicle once.

Note. The drift of *p*H during the course of the experiment was as follows:

Test A. *p*H 5·4 to 5·6
B. *p*H 6·0 to 6·2
C. no change.

Table of counts in hundreds of millions per c.c.

Hours	*p*H 5·4	*p*H 6·0	*p*H 6·8
0	102·0	124	124
20	7·7	89	80
26	6·3	56	68
32	6·0	65	67
42	1·9	34	39
52	0·97	23	34
66	0·07	4·9	2·4

At this point, the poor keeping qualities of the *Brucella* vaccine in the Indian field were reconsidered. In an earlier experiment (Table XL) counts, made on vaccines sent to the Military Veterinary Laboratory, Lahore, stored for varying periods at average temperatures of 71° and 77°F. and returned to Mukteswar, compared very unfavourably with counts on a control vaccine stored at a constant 79°F. in the Institute. Therefore, as temperature alone was clearly not to blame, it appeared that death might have been due to (i) changes in temperature during transit or (ii) shaking during transit.

In experiment V, Cotton's strain 19 was suspended at density 5 (Brown's scale) in the optimum vehicle evolved in Experiments I to IV (*i.e.*, 0·85 per cent NaCl in glass-distilled water, buffered with M/50 Sorensen's phosphates to *p*H 6·4, with the addition of 0·1 per cent tryptose). This suspension was divided into three parts; one portion was stored continuously at 42°C., a second sample was moved from one incubator to another, so as to vary the temperature of storage as much as possible, whilst roughly imitating the diurnal temperature changes on the plains (see schedule on the reverse of Fig. 9.) A third sample was shaken from 10 a.m. to 4-30 p.m. and throughout the night was stored at 42°C. without agitation. The shaking was achieved by placing the suspension in a bottle which contained 3 parts of air and 1 part of vaccine and attaching the bottle to a rotor moving in a plane set at approximately 30° to the vertical. In this way the vaccine was precipitated from end to end of the bottle, twice in each revolution. The speed of rotation was 120-150 r.p.m. As may be seen from an inspection of Fig. 9, the bacterial death-rate was not strikingly affected by shaking and not at all by variations in temperature. A puzzling result, for not only is it at variance with the inference drawn from the mortality occurring during transit in India, but it also disagrees with the findings of Mitchell and Moore [1942], who, by means of counts taken before and after various journies, demonstrated the lethal effect of trnasportation on *Brucella*.

Nevertheless, it must be recalled that in the foregoing experiment the control remained for the whole time at 42°C. whilst, during the shaking periods, the shaken sample was at bench temperature. Thus, as the two mortalities are almost the same, it is to be supposed that bacterial destruction due to shaking at say 22° to 25°C. equals that due to storage at 42°C. Moreover in this experiment the

optimum vehicle was used, whilst the vaccine sent to Lahore was made with saline. Accordingly, two more experiments were made. In the first, a saline control was kept continuously at 42°C and both an optimum and a saline sample were shaken, as before (during the day at room temperature and stored during the night at 42°C). Another saline sample instead of being shaken was jerked. Jerking was arranged by securing the bottle containing the vaccine to a strip of copper sheet anchored at one end only ; the free end of the strip was within reach of a four armed rotor (r.p.m.=150) adjusted so that each arm struck the sheet in passing. The sheet thus became a slow vibrator and transmitted vibrations to the vaccine in a manner reminiscent of railway-travel in India.

In this experiment, the greatest death-rate occurred in the saline control ; the least in the optimum shaken sample, whilst after the first 24 hours the comparative rate in the jerked saline sample was considerable (Fig. 10).

In the other experiment (Fig. 11), a saline suspension was kept at bench temperature continuously, while a saline and an optimum sample were shaken at the same temperature without interruption for 72 hours. In this experiment there was a noticeably greater death-rate in both the shaken samples than in the control.

A review of the preceding work forces the conclusion that temperature of storage, age and agitation cause *Brucella* vaccines to deteriorate and while the greatest individual contribution towards this end is undoubtedly temperatures higher than about 70°F. the cumulative effect of all is very considerable. Further, in all tests, the optimum substrate evolved in the earlier work proved slightly better in most environments, and very much better in extreme environments than did the saline. Therefore, the technique recommended for the preparation and issue of *Brucella* vaccines in India is as follows.

The vaccine strain is to be sown on liver agar *p*H 6·8-7·0 and incubated for 48 to 72 hours at 37°C. The growth is then to be washed off in a small measured quantity of optimum vehicle (say 25 c.c. per roux flask). The yield from sufficient flasks is pooled (one flask is generally sufficient to produce 100 to 150 c.c. of finished vaccine) and a small sample of the pool is tested to get the dilution factor required to give a density of tube 5 or 6 on the Brown's scale. The pool is diluted accordingly and bottled.

Where possible, vaccine should not be issued between April and September, but, if it must be used during this period, not more than 7 to 10 days should elapse between the despatch from Mukteswar and the cancellation date marked on the bottle. In winter, when routine vaccination should be practiced, a period of validity of three weeks might be permitted, but, in every event, the instructions should urge the earliest possible use of the vaccine.

It is also permissible to store vaccine at Mukteswar at zero degrees C. for one or two months between preparation and issue ; it may be noted further, that the easily obtained and less costly gelatin may be substituted for tryptose in the preparation of the optimum vehicle.

THE PRESENT POLICY OF VACCINATION IN INDIA

At present, vaccination against Brucellosis is recommended when eradication by hygienic control seems improbable, or it has been tried and failed. As a general guide, vaccination need only be considered when farms have an abortion rate of more than 5 per cent of the breeding stock per annum and conditions adverse to hygiene control (p. 157) exist. The class of stock to be vaccinated may be selected to suit local convenience, the primary object being to protect all healthy females both existent and to come. The first group of vaccinees that usually presents itself is existing uninfected adults and, because these must be inoculated when not in-calf, the work becomes troublesome as it entails the vaccination of two or three cows at a time as they accumulate after calving. It usually also means vaccinating in-milk animals, which causes a small but not, it is to be noted, permanent drop in the milk-yield. Therefore, if only a few uninfected adults remain in a herd, it is for consideration, whether it is worthwhile vaccinating them. Where, however, a number remains, vaccination

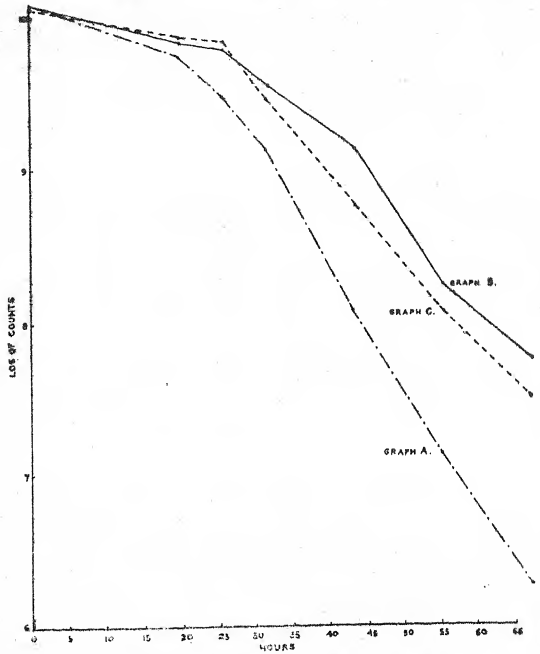

FIG. 6. * The death-rate of *Brucella* in variously composed substrates.

A. in distilled water

B. in 0·85 per cent pure NaCl

C. in complex saline

All substances were buffered with Sorensen's M/50 phosphates to *p*H 6·0.

Experimental conditions in fig. 6

Vehicle. Glass distilled water was used in all cases. The composition of the complex saline used in C. was as follows:

NaCl 0·9 per cent
$CaCl_2$ 0·024 per cent
KCl 0·042 per cent
$NaHCo_3$ 0·01 per cent
$MgCl_2$ 0·1 per cent

*For further details and table of bacterial counts see page 171

Storage. The temperature of storage was 42° C.

Suspension. The suspension was prepared from a 48 hours old culture of Cotton's strain 19, washed once by centrifugation and resuspension. Its density was 5 on the Brown's scale.

Note. The *p*H of the substrate drifted during the test as follows:

Test A. *p*H 6·0 to 6·2

B. *p*H 6·0 to 6·2

C. *p*H 6·0 to 6·4.

Table of bacterial counts in hundreds of millions per cc.

Hours	Distilled H_2O	Saline	Complex saline
0	117	118	110
20	55	66	70
26	30	60	68
32	14	35	29
44	1·25	14	6
56	0·14	1·9	1·14
68	0·02	0·6	0·34
92	0·0	0·08	0·06

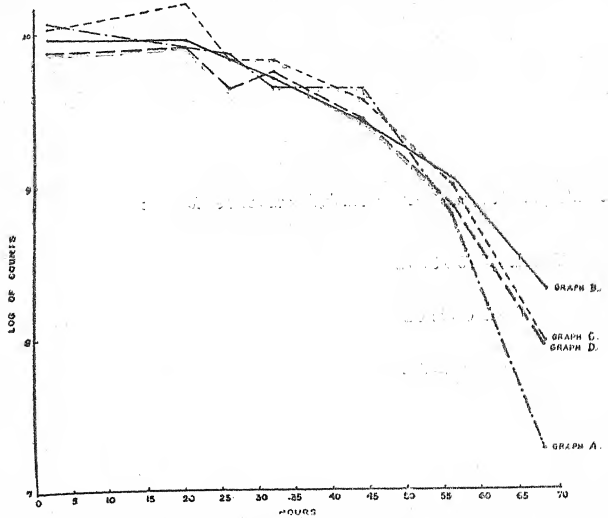

FIG. 7. The death-rate of *Brucella* in variously composed substrates:

A. Pure NaCL 0·85 per cent buffered *p*H 6·4
B. Pure NaCL 0·85 per cent buffered *p*H 6·4 plus 0·1 per cent tryptose.
C. Pure NaCL 0·85 per cent buffered *p*H 6·4 plus 0·1 per cent gelatin
D. Pure NaCL 0·85 per cent buffered *p*H 6·4 plus 0·1 per cent glycerine

Experimental details of fig. 7

Suspension. The suspension was prepared from a 48 hours culture of Cotton's strain 19 at density 5 on Brown's scale. It was washed once by centrifugation and resuspension.

Storage. At 42° C.

Table of bacterial counts in hundreds of millions per cc.

Hours	Test A	Test B	Test C	Test D
0	120	94	106	75
20	81	92	157	81
26	70	68	68	44
32	44	50	65	55
44	42	25	35	26
56	6·3	10·4	9·7	7·0
68	0·19	2·1	0·97	0·93

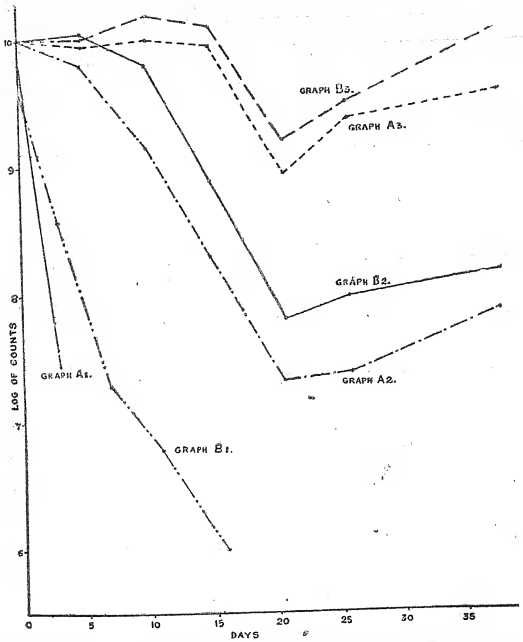

FIG. 8.* The keeping qualities of *Brucella* at three different temperatures of storage and in a simple and an improved substrate :

A. Simple Saline substrate

B. Improved substrate

1. Storage at 42°C

2. Storage at 30°C

3. Storage at 0°C

Details of experiment in fig. 8

Suspension. The suspension was prepared from a 48 hours old culture of Cotton's strain 19, at density 5 on the Brown's scale.

Substrate. The simple substrate consisted of 0·85 per cent common salt in distilled water. The improved vehicle was made up as follows.

NaCl pure 0·85 per cent

Tryptose 0·1 per cent in glass distilled water buffered with Sorensen's phosphates M/50 to *p*H 6·6.

* For table of bacterial counts see overleaf.

Bacterial counts in hundreds of millions per cc.

Days	A1	B1	A2	B2	A3	B3
0	63	38	103	104	103	104
3	0·20	3·6	..	..	..	..
5	..	..	68	109	93	104
7	..	0·14	..	..	..	..
10	..	..	14	64	101	149
11	..	0·06	..	..	..	..
15	..	..	3·5	8	91	123
16	.	0·009	..	..	..	..
21	..	..	0·2	0·63	8·9	16·4
26	..	..	0·23	0·93	24·3	32·4
38	..	..	0·7	1·44	37	117

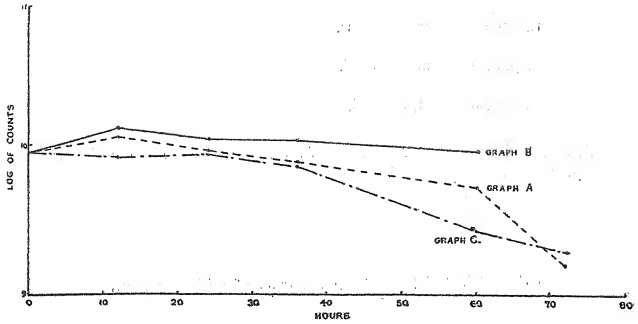

FIG. 9. *The death-rate of *Brucella* in various environments.

A. Stationary at 42°C

B. At changing temperature

C. Shaken at room temperature during the day

*For details and table of bacterial counts see overleaf.

Experimental details of Fig. 9

Suspension. Prepared from a 48 hours old culture of Cotton 's strain 19, at a density of 5 on the Brown's scale.

Substrate. Glass distilled water containing 0·85 per cent. pure NaCl and 0·1 per cent. tryptose, buffered with Sorensen's phosphate M/50 to *p*H 6·4.

Storage. Test A. (Control) stationary at 42° C.

Test B. 10 a.m. to 12 noon at 37° C.

12 noon to 4 p.m. at 42° C.

4 p.m. to 8 p.m. at 30° C.

8 p.m. to 8 a.m. at 0° C.

Test C. Shaken at room temperature from 10 a.m. to 4·30 p.m. and stationary at 42° C during the night.

Table of bacterial counts in hundreds of millons per cc.

Hours	Test A	Test B	Test C
0	88	88	88
12	121	131	84
24	94	113	87
36	77	110	77
60	51	91	26
72	15·9	..	18·5

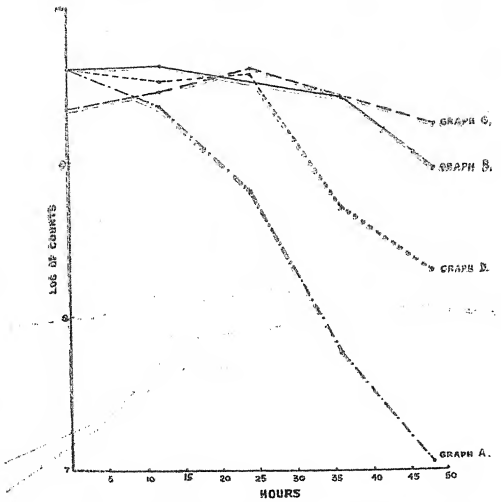

FIG. 10. The death-rate of *Brucella* in different environments.

Experimental details of fig. 10

Suspension. Prepared from a 48 hours old culture of Cotton's strain 19, at density 5 on the Brown's scale.

Substrates. In tests A, B and D 0·85 per cent common saline.

In test C as in fig. 9.

Storage. Test A (control) stationary at 42° C.

Tests B and C shaken, and in Test D jerked at room temperature from 10 a.m. to 10 p.m. and stationary during the night at 42° C.

Table of bacterial counts in hundreds of millions per cc

Hours.	Test A	Test B	Test C	Test D
0	39	39	22	39
12	23	42	28	33
24	6·3	..	39	37
36	0·59	26	25	..
48	0·11	6·9	17	1·8

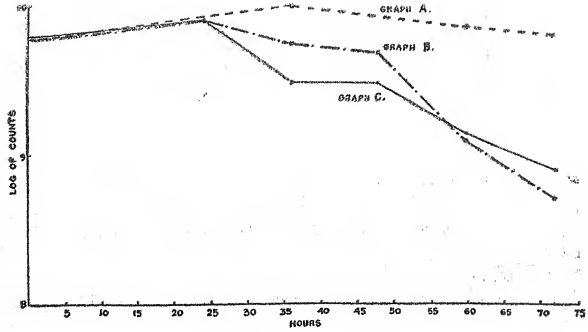

FIG. 11. *The death-rate of *Brucella* in different environments, etc.

Test A. Unshaken at room temperature (control) common saline

Test B. Shaken at room temperature, common saline substrate

Test C. Shaken at room temperature, improved substrate

*For details and table of bacterial counts see page 181.


PESHAWAR
LAHORE
QUETTA
DELHI
LUCKNOW
PATNA
SHILLONG
CALCUTTA
NAGPUR
CUTTAK
BOMBAY
MADRAS
MYSORE

FIG. 12.

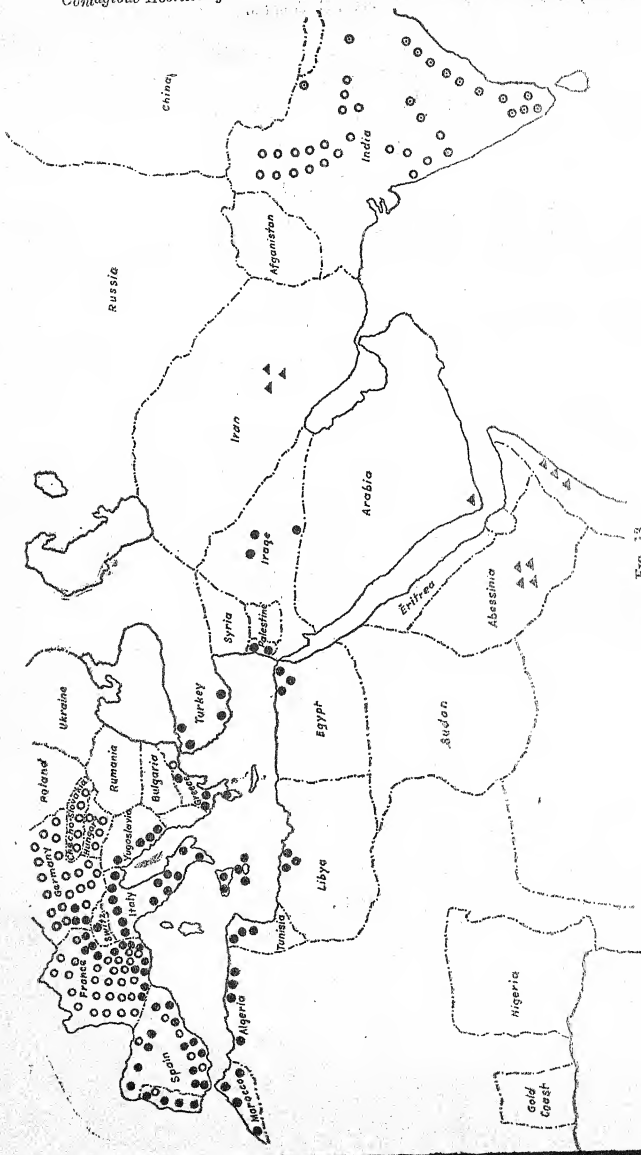
China
India
Russia
Afganistan
Iran
Arabia
Iraqe
Syria
Palestine
Eritrea
Abessinia
Ukraine
Turkey
Egypt
Sudan
Poland
Rumania
Bulgaria
Yugoslavia
Germany
Italy
France
Spain
Algeria
Tunisia
Libya
Morocco
Nigeria
Gold Coast

FIG. 13.

Experimental details of fig. 11

Suspension. Prepared from a 48 hours old culture of Cotton's strain 19, at a density of 5 on the Brown's scale.

Substrate. Tests A and B. 0·85 per cent common salt in distilled water.
Test C. as in fig. 9.

Storage. Test A. At bench temperature, unshaken.
Test B. Shaken at bench temperature.
Test C. Shaken at bench temperature.

Table of bacterial counts in hundreds of millions per cc.

Hours	Test A	Test B	Test C
0	58	58	60
24	..	79	79
36	95	55	30
48	..	47	29
60	70	13	13·4
72	57	4·8	7·2

cannot be omitted, but the owner should be fully informed of the position. There remain two other groups of possible vaccinees, viz., heifers, which become eligible for inoculation at any time during the six months preceding the anticipated age of first oestrus, and calves between 4 to 8 months of age. It is for decision which of the classes, both now, and in the future are going to be most convenient to vaccinate and, in deciding this, the following points should be borne in mind : (1) that calves are more easily handled than heifers—with wild stock an important point ; (2) that, on the whole, a better immunity can be hoped for in heifers and (3) that if the decision is to vaccinate calves, then the existing heifers and all young stock over eight months must be protected. This must be done by vaccinating heifers until the first batch of calves vaccinated at 4-8 months are coming through. Other minor points, such as ease of access to and manipulation of future batches of vaccinees are also worthy of consideration. Having settled on one or other group of on-coming young stock, they should be vaccinated in batches, as they accumulate, either quarterly or half-yearly.

It is difficult to advise on the period for which vaccination should be continued, but, in deciding on its discontinuance, factors affecting the introduction and transmission of new disease (p.131) should be duly weighed, abortions should be less than 1 per cent per annum and, if a non-agglutinin-producing vaccine has been used, the adult stock should be tested and found negative before control measures are withdrawn.

Difficult types of farms to deal with are either military farms, where of necessity, stock is being continually interchanged or farms, where replacement animals are purchased. In the former, a universal routine vaccination of young stock and purchases might suffice to avert disease but, in the latter, attention must be given to the source of purchases, in relation to endemic areas, which should be barred. Animals should be bought subject to their passing the quick test and afterwards vaccinated as quickly as possible.

Field trials of vaccination

Up till now, *Brucella* vaccine has been used in several south Indian villages, in some four or five private farms and in five military establishments. As regards villages, not only is it too early to

attempt to get results, but records, other than a very rough statement derived from the village spokesman, will probably never be available. Turning to farms, one private recorded herd was vaccinated as early as November of 1941 and here alone is it possible to review the results of two completed breeding seasons. The vaccinated animals were mainly empty adult Punjabi buffaloes and the injection consisted of 50 thousand million organisms of Cotton's strain 19, suspended in 5·0 c.c. of saline. A single dose was given subcutaneously. The abortion rate for the six prevaccination years 1936-41 can be seen on page 131 in (Table XXI); the rate since 1941 is given in (Table XLIII).

TABLE XLIII

Post-vaccination abortion rates

Animal group	Abortions/ pregnancies per cent per annum	Pregnancies completed
Non-reacting vaccinees	1·56	32
Random vaccinees	4·69	32
Positive reactors (not vaccinated)	4·17	12
Pregnant not vaccinated	4·72	159
Non-reacting controls	4·65	24

Overall mean annual abortion rate for two post-vaccination year = 3·96 per cent

It appears then, that an overall mean annual rate of 21 per cent in the prevaccination years dropped to an overall mean of 3·96 per cent in the two post-vaccination breeding seasons, while the lowest abortion rate was 1·56 per cent in the non-reacting principal vaccinees. Nevertheless, it is debateable how much this drop is due to the vaccination and how far it may be attributed to a natural cessation of the endemic. Unfortunately, a study of the abortion rates in the various groups reveals little, for, while it is true that the principal vaccinees show one third less abortions than the controls, the rates in all other groups are uniformatively alike, worse still, the rates in the very large batches of unvaccinated animals were very much lower than the lowest rate for the preceding six years. It is hard to believe that the protection of 77 animals so reduced the concentration of disseminable contagion that most of the remaining 150 or so animals escaped infection, and the result is as bewildering scientifically, as it is gratifying in practice. At the most, but a poor case can be made out for the vaccine, while, there is much to suggest that the endemic might well have ended spontaneously; for instance, no new buffaloes had been purchased for some three or four years, while the home-bred new-entry were few. It is easy to suppose, therefore, that after a 20 per cent abortion rate for six years much of the herd had become naturally immune.

However, as a result of renewed confidence inspired by the fewer abortions, the owners are buying 100 new adults in 1944; these animals are to be vaccinated and the real test will come when they commence to breed.

Another fairly large scale trial of vaccination has been made in the young cow-stock of five military farms Animals were inoculated in two age groups viz., at 4 to 8 months and 18 to 24 months respectively. A single subcutaneous injection was given of 5·0 c.c. of Cotton's strain 19 suspended at a density of 7 to 8 on the Brown's scale. As yet, insufficient results have accumulated to permit the formation of an opinion on this work, but such records as are available are analyzed in Table XLIV, p. 183. They are at present unworthy of discussion.

TABLE XLIV

Results of the vaccination of calves and heifers in military farms

Farm	Vaccinees age group 4—8 months								Vaccinees age group 18—24 months								Calving results of non-vaccinated controls					
		Agglutination results						Calving results		Agglutination result						Calving results		Group 4 to 8 months			Group 18 to 24 months		
	No. done	1st test				2nd test		Normal	Abnormal	No. done	1st test				2nd test		Normal	Abnormal	No. kept	Calvings		No. kept	Calvings	
		+++	++	±	—	++	—				+++	++	±	—						Normal	Abnormal		Normal	Abnormal
I	26	24	2	—	—	11	9	—	1	27	26	1	—	—	19	3	16	1*	27	—	1	25	14	1
II	29	14	5	6	—	13	10	—	—	13	13	—	—	—	13	—	2	—	29	—	—	13	—	—
III	37	36	1	—	—	24	8	—	—	24	24	—	—	—	24	—	—	1	37	—	—	23	—	—
IV	28	20	6	1	1	12	9	—	—	48	44	2	—	—	36	—	—	—	27	—	—	48	—	—
V	27	13	8	5	—	12	13	—	—	25	22	3	—	—	19	6	—	—	27	—	—	25	—	—
VI	32	25	3	3	—	8	6	—	—	—	—	—	—	—	—	—	—	—	31	—	—	—	—	—
Total .	179	132	25	15	1	80	55	—	1	137	129	6	—	—	111	9	18	2	178	—	1	134	14	1

+++ = Strongly positive
++ = Weakly ,,
± = Doubtfully ,,

— = *Nil*
* = Stillborn

Section VIII. Specific abortion, Other than Brucellosis

(Abortions caused by specific primary genital-infection, other than Brucellosis)

During the course of the scheme, a constant vigilance has been maintained for evidence of specific primary genital infections, which, Brucellosis apart, are known to cause abortion; those commonly described are (i) Trichomoniasis, (ii) Listerellosis, (iii) Vibrio infections, (iv) *Salmonella* infections, to which may be added one or two bacterial diseases suspected of having the occasional ability of causing abortion. It may be said at once that, in India, no epidemic abortion has been encountered which could be attributed to any of these infections; but the following notes on each are appended.

Trichomoniasis

Infection by *T. foetus* has been suspected in occasional cases of bovine abortion or sterility in south India. Suspicions have generally been aroused by the co-existence of purulent vaginal discharges with abortion—one of the characteristics of this infection. The writer has, from time to time, come across such discharges, notably in buffaloes, but he has never been able to trace the complete typical syndrome with all its manifestations of vaginitis, abortion, sterility and above all, plurality of infection obviously transmitted by the bull. In these sporadic cases, and also in the case of three herds suffering from abortion, numerous slides of cervical washings have been made, but in no case, could the disease be diagnozed. Further, this seems to be the experience of other interested workers, notably in south India. Although *T. foetus* has never been discovered in slide examinations, on two occasions, slides of vaginal washings of from aborted buffaloes have revealed a heavy infestation with sarcocysts; but, as numerous preparations from other co-aborting animals did not reveal similar infestation, the incident could not be accepted as significant.

The methods used for diagnosis of protozoa in the vaginal secretions were as follows: (1) direct microscopic examination of freshly-made, wet, unstained smears (this is probably the best method, provided the apparatus is available on the spot and the appearance of the live trichomonad is familiar to the operator); (2) microscopic examination of smears made from the centrifuged saline washings of vaginal swabs; (3) the seeding of special culture media (media kindly supplied by Dr H. N. Ray of the Imperial Veterinary Research Institute, Mukteswar-Kumaun, United Provinces, with suspected material; (4) considerations of epidemiology and clinical history. At one time, plans were formed to develop the agglutination test for *T. foetus* in India, but, as type-strains could not be transported from Europe, in the conditions or delay imposed by the war, the idea had to be abandoned.

Salmonella listerella and vibrios

In plating specimens from aborting animals for the isolation of *Brucella*, a record has been kept of common commensals; but none has in any way resembled *Vibrios* or *Salmonella*, and with the possible exception of the case described in the next paragraph none has resembled *Listerella*. On certain occasions, serum from aborting sheep and goats has been tested for agglutination against *Salmonella abortus ovis*, but in no case was the titre diagnostically significant. This failure to discover, either these organisms, or trichomonads does not, however, imply that they are wholly absent from India, but it does suggest that their incidence or pathogenisis is insufficient to render them consipicious to the diagnostician.

A possible new aetiology of goat abortion

The sole exceptions to the foregoing generalization are (1) the *Listerella*—like strain mentioned on page 191 together with a nearly identical strain isolated from the stomach contents of a bovine foetus, aborted in Dehra Dun. These strains are denoted X31 and X39 respectively, while sub-strains recovered from animal inoculation, are given the number of their host prefixed by the letter X.

As both these strains were isolated during the last few months of the scheme and, as great difficulty was experienced in persuading them to survive at all, let alone submit to tests on artificial media,

they have been very scantily examined. Again, on account of the extreme difficulty of obtaining pregnant goats for animal-inoculations and of preventing the few obtained from dying of pneumonia in the rigours of the Kumaun winter, the results of animal passage are too few to be convincing. For what they are worth, however, they are very briefly appended.

The organism is a small gram-positive cocco-bacillus or bacillus, reminiscent of the short form of *Listerella*, but, unlike *Listerella*, long forms have not yet been observed and pleomorphism is slight. The organism is believed to be sluggishly motile, but growth is so feeble at bench temperatures that inspection during the first 72 hours is almost fruitless. Spores are not formed. Growth at 37°C is almost equally problematical in broth or on agar and unless strains are passaged every three days they will sooner or later be lost. ACO_2 atmosphere does not improve matters. Comparative growth has been observed on 2 per cent tryptose agar, liver agar, and 1 per cent glucose agar and in 2 per cent tryptose and 2 per cent, 1 per cent and 0·5 per cent glucose broth. One per cent glucose seems to be very much better than other concentrations while 2 per cent tryptose is a very good substitute. On solid media, colonies are pin-point to small pin-head, translucent, sparkling and beadlike. They neither enlarge with time nor tend to coalesce. So far, isolation cultures have shown sparce colony growth only. The continuous growth, seen on established cultures, is so faint as to be only discernable by the closest scrutiny, while manipulating the slant in transmitted light. For a day or two, it appears as little more than a faint fogging on the surface of the agar, which may be confused with a patch of dried condensation. This wraith-like appearance on solid media is characteristic and although well-established strains occasionally get slightly denser, as a rule, the culture dies before it can thicken. The growth in broth produces a uniform faint turbidity, part of which, in well established cultures, deposits in the form of light flakes.

On none of the media used, can growth be expected before the second or third day of incubation and even well adapted strains may fail to emerge before the fourth. At any time a strain may die for no apparent reason and to obviate this mishap it is desirable to maintain cultures in 2 per cent tryptose or 1 per cent glucose broth and to subculture twice weekly by pipetting a substantial quantity from the old to the new tube. Before this system was developed several promising lines—X31 amongst them were lost, and at one time it was thought impossible to maintain even a single example.

Table (XLV) gives the results of the usual biological tests, which were compared with Miss Barber's [1939] findings for *Listerella*. On the whole, there is considerable dissimilarity and to clinch matters, cross agglutination with *Listerella* does not occur.

TABLE XLV

The biological reactions of X strains

——	Listerella	X 31	X 39
Glucose	+	+	+
Inulin	—	0	—
Maltose	+	—	—
Raffinose	—	0	+
Salicin	+	—	+
Lactose	± late	± late	± late
Mannite	—	0	—
Rhamoose	+	—	—
Sorbito	—	—	—
Dextrol	+	+	+
Sucrose	± late	+	+
Trehalose	+	± late	+
Gelatin	—	—	—
H_2S	—	—	0
Nitrates	0	—	—
V. P.	+ weak	—	—
Methyl Red	×	—	—
Indol	—	—	—
Litmus milk	+ trace	—	—

+ = Acid, no gas. — = No acid, no gas. 0 = test not done.

Both strains were non-pathogenic for mice, guinea-pigs, and rabbits; thus again differing from *Listerella;* but in pregnant goats one or two pathogenic effects were noted that can hardly be ignored. They are schematized in Table (XLVI).

TABLE XLVI

The injection of pregnant goats with X strains

Goat No.	Inoculum and route	Results
380	X.39 (5·0 c.c. of a 48 hours broth culture, intravenously).	Aborted within 24 hours; culture of foetal stomach contents gave X-like strain. Other foetal tissues sterile. Hæmocultures on 8th and 14th day sterile vaginal swabs on 12th, 13th, 14th and 15th days gave cultures of X-like organisms. Strain isolated denoted X380.
341	X39 (5·0 c.c. of a 48 hrs. broth cultures, intravenously),	None, in two months.
430	X39 (5·0 c.c. of a 48 hrs. broth culture filtrate, intravenously).	None, in two months.
456	X380 (5·0 c.c. of a 48 hrs. broth culture, intravenously).	Died 10th day with symptoms of fever and pneumonia. Culture made from placenta and foetus yielded a typical X organism, denoted X 456.
504	X456 (5·0 c.c. of a 48 hrs. broth culture, intramuscularly).	Aborted within 48 hours. Foetal cultures sterile. Died 6 days after injection with enteritis and metritis.
593	X456 (5·0 c.c. of a 48 hrs. broth culture, intravenously).	Died five days later with nascent pneumonia. Organism typical of X strains isolated from placenta. Culture denoted X 493.
332	X31(5·0 c.c. of 48 hrs. broth culture, intravenously).	Aborted after 20 days. Foetal culture negative for X types. Developed purulent metritis. Died 10 days after abortion. No X-type could be isolated.

Out of six pregnant goats receiving viable X strains, only one remained unaffected (No. 341) while three aborted and four died. From three affected goats an X-like strain was recovered in pure culture, in each case from the genital tissues. The remarkable and baffling point is that two of the three abortions occurred within 48 hours of the administration of the injection; in one case in the night following and in the other during the second night following inoculation. It is scarcely believable that these early abortions could be the direct result of bacterial invasion, but it is perhaps possible that this organism is a fairly common inhabitant of the goat and that the injections precipitated a crisis of an otherwise subclinical infection. In this respect it has already been noted, how little is required to provoke a goat to abortion. It is perhaps unfortunate that no tissues of the dead goats, other than genital, were cultured. Pneumonia is fairly common in goats and at that time it was not associated with the injections. In retrospect, however, it does not seem improbable the X organism may have been a cause of the pneumonia, and in fact be concerned in goat pneumonia in general. On

the present evidence it would be folly to suggest, that a new cause of goat abortion has been discovered, but it does seem desirable that when future cases of obscure epizootic goat abortion are being investigated, organisms of the type described should be diligently sought. Further, in view of some of the similarities between goat and buffalo abortion, it is not impossible that X strains might be encountered in the latter species also.

SECTION IX. NON-SPECIFIC ABORTION

(Abortions not demonstrably caused by specific primary genital infection)

Cows

Although non-specific bovine abortion is infrequent in India, in almost every herd, occasional abortions occur that are not due to obvious disease. The incidence of such casualties is from 0·5 per cent to 2·0 per cent *per annum*, their appearance is sporadic and their cause is difficult to discover. Many attribute these abortions to injuries received in fights, to careless driving through door-ways and gates and to slipping in railway waggons or on ill-faced cow-house floors. Such explanations are plausible, but the fact remains that a perfectly healthy cow is capable of sustaining a considerable amount of rough handling (*e.g.*, in casting) without aborting; whilst, if the analogy be tenable, a mare far advanced in pregnancy, may roll for hours in colic and fail to abort.

Plant-poisoning is another favourite retreat of the baffled diagnostician, but, whenever this explanation has been proffered during the present work and where malpractice could be ruled out, the writer has not known a single instance supported by scientifically acceptable proof, while, in the cases where the fodder has been analyzed, the reports have always shown it to be harmless. However, in one occurrence of several abortions in a jail herd in Bengal, malpractice was definitely suspected, firstly because all abortions occurred outside the jail wall, in an easily accessible place, secondly because most of the animals died during delivery, and thirdly because pregnant animals of the same batch taken inside the jail failed to abort. The local authorities suggested that there was a plant known in the district which if administered to cattle caused poisoning and abortion, whilst another possibility is trauma caused by inserting sticks or other objects *per vaginam*. As, however, the cases were apparently not very carefully examined at the time of the occurrence, the real explanation will probably never be known. Whatever the cause, it is quite out of the ordinary for a majority of animals to die from an apparently uncomplicated outbreak of abortion and this can be regarded as a unique and irrelevant incident.

A far more genuine cause of abortion in which the genitalia are not primarily involved, is an attack of a severe infection such as rinderpest, or foot-and-mouth disease, and although the sequel of abortion is not so common in cows, as in buffaloes, sheep and goats, one clear case was encountered during the present work. A combined attack of rinderpest and hæmorrhagic septicaemia appeared to be the inciting stimulus, the abortions occurring nearly simultaneously in several Brucella-free convalescents. There seems good reason to suppose therefore, that many otherwise inexplicable abortions could be attributed to previous sickness or physical upset were the history of the case but known.

Deficiencies of diet have often been suggested as causes of abortion and conjectures of this sort again provide a smoke-screen for ignorance. It is a truism that abortions are commonest in scientifically fed herds and rarest amongst half-starved village animals. This may perhaps be explained on the grounds that scientifically fed animals are also almost always forced, whereas, the village cow can adjust her output to changes of environment. Nevertheless, although several workers have more or less associated a deficiency of various food factors with abortion, it has yet to be shown that, in ordinary circumstances, the lack of any particular item of diet has caused wholesale abortion in an otherwise healthy herd. That certain deficiencies, coupled with forcing, may predispose to infection is by no means questioned, but academic proportions such as these must be placed on a rather more practical basis before they can be treated seriously in Indian field practice.

Buffaloes

Throughout part I, constant allusion has been made to certain peculiarities of buffalo abortion whose specificity was held in question. The salient points recapitulated are (i) sharp epidemics of buffalo abortion can often be connected with preceding heavy rains, (ii) when these epidemics are primary and where the buffaloes are not co-habiting with cattle, it is unusual to find more than a few doubtful *Brucella* reactors amongst the affected buffaloes, (iii) in exacerbations of minor endemics, when buffaloes are co-habiting with Brucella-infected cattle, the reactor rate in buffaloes is about half of that in cattle and the serum titres of the buffaloes are low compared with those of the cows, (iv) towards the end of prolonged outbreaks of abortion in buffaloes *Brucella* reactor rates have been noted equal to the normal rate in cows irrespective of whether the buffaloes are with cows or not, (v) buffaloes seem slow to contract Brucellosis from co-habiting cows, (vi) at least one epidemic of buffalo abortion has been investigated in which unusual symptoms have been noted.

It seems that these anomalies can be explained by one of three postulates, viz., (1) that the abortions are the result of some effect of the rain, e.g., chill, of depression (2) that they are caused by an atypically manifested Brucellosis, exascerbated by rain, (3) that they are due to an unknown rain-invoked infection. The possibility of the chilling effect of rain being the unaided cause of abortion seems, at first sight, remote, for there is usually a lag period of some weeks between the rains and the epidemic. As, however, a similar lag is sometimes observed when abortions are plainly the sequel of fevers, this possibility cannot be entirely discarded. Indeed, on deeper enquiry, this postulate becomes more attractive, in as much as the two species in which unexplicable abortions connected with rains occur, are the buffalo and the goat, animals which are liable to be severely affected by chilling. It might be further argued that continuous rainfall enhances this effect by depressing the buffalo's very sensitive metabolism over long periods. Brucellosis could be accepted as the cause, were *Brucella* reactors constantly and adequately present in all epidemics, and the part played by the rain could be explained by the hypothesis of 'a better infective dose being transmitted by fluids' (p. 134). But, if, in the face of so many poor agglutination reactions, an aetiology of Brucellosis is insisted upon, then it must be conceded that, in primary outbreaks, the *Brucella* agglutination response of buffaloes is bad. There is considerable data to be found throughout this paper which adds support to this supposition; furthermore, it is irrefutable that Brucellosis is at least intercurrent in many epidemics, and, while, in this disease, the carrier-role of fluids seems a reasonable one, the preserving value of humidity on the contagion has been demonstrated.

Finally, if neither of the preceding suppositions be tenable, then certain sporadic primary outbreaks of buffalo abortion must be due to an unknown waterborne infection, for this is the only other solution which fits the facts. Nevertheless, it remains a solution without an aetiology, and to demonstrate the difficulty of securing evidence of another causal factor the following case report is cited.

In an epidemic of abortions among buffaloes co-habiting with and presumably infected by cattle, the *Brucella* reactor rate in buffaloes was half that of the cows, the rate in the cows being normal. In the buffaloes only, there were the following symptoms, which disagreed with the *Brucella* syndrome, (1) abortions occurred much earlier in pregnancy than was normal for *Brucella* infected buffaloes, i.e., at three months term (2) a proportion of buffaloes were emitting a purulent vaginal discharge, (3) a few necrotic ulcers the size of a shilling were seen on the vaginal mucosa. Sabotage, plant-poisoning and the sequel of vaccinations or febrile infections could be ruled out. The vaginal secretions were examined for protozoa by the usual means and for bacterial infection by numerous small animal injections and direct cultures. But no pathogen, including the *Brucella* microbe, could be found. It is to be noted that the outbreak was preceded about one month earlier by exceptional rains.

For the present, therefore, it is not possible to adjudicate between the three hypothesis, and plainly, experiment should determine the true course of Brucellosis in buffaloes and the effect of their

being chilled while pregnant. At the same time, further field investigation is needed in search of the postulated pathogen.

In the face of so much incertitude it is pleasant to be able to recall that buffaloes are definitely known to abort after an attack of a severe fever or systemic disturbance, e.g., rinderpest, antirinderpest injections, haemorrhagic septicemia, or foot and mouth disease and the likelihood that they are more liable to this accident than cows adds credence to the conjecture that abortions may be also caused by chilling during rainfall.

Sheep and goats

Although abortion due to Brucellosis seems to be quite rare in the village sheep and goats of India, abortion associated with extra-genital disease is common. Indeed it seems possible that these animals will abort as the result of any disease which seriously impairs their health, e.g., pneumonia, rinderpest or rinderpest pneumonia, contagious pleuro-pneumonia (goats), wah, pox and even parasitic infestation. On the whole, goats appear to be worse offenders than sheep; indeed the writer is of the opinion that goats will sometimes abort as the result of the slightest interference even one so trivial as an intravenous injection. According to some reports, the rate of abortion at such times may be extremely high and even 70 to 80 per cent has been suggested. Whenever blood testing has been possible in connection with these outbreaks, the results have been consistently negative to Brucellosis and *Salmonella* infections.

As in buffaloes, so in goats there are certain abortion epidemics of obscure aetiology. Unhappily the majority of these have occurred long before they can be investigated and as a rule no recorded history is available. One government herd, however, has been investigated and reinvestigated for more than a decade and while, like those of the last sub-head, the results of their investigations are largely speculative; as they not only typify some problems of goat abortion, but also parallel the enigmas of buffalo abortion they merit a short critical review.*

The herd in question was founded in the summer 1928, mainly with in-kid goats purchased from around Gujranwala but about 20 local goats were already on the farm as early as April of that year. In the same autumn one local and four purchased goats aborted, and abortions have continued to the present day. Both then and since, undoubted Brucellosis has been prevalent in a large cow-herd grazing the same forest, and, although the occurrence of Brucellosis in some at least of the goats is irrefutable, many attempts have been made to prove that the actual abortions are due to some other cause. The reasons for this are threefold viz., (1) with the exception of one dubious record, many careful attempts to isolate *Brucella* from the goats have failed. (2) The serum agglutination test has given irregular and, at times, meagre proof of *Brucella* infection. (3) At times, aborting goats have shown symptoms of other diseases, notably metritis and mastitis, thus suggesting a different aetiology.

To anyone familiar with the difficulties of isolating *Brucella* from goats in India, item (1) will appear insignificant, and if, indeed, for many valid reasons, it has been impossible to isolate *Brucella*, it has been equally impossible to secure a substitute pathogen, or even to offer the least vestige of a substitute pathology. A new pathology might, at first sight, be thought to appear in item (3) in as much as, out of 66 goats aborting between 1929 and 1935, 17 were stated to have died of metritis within a month or so of parturition: on the other hand no such deaths have been reported since 1935, and none occurred in a big abortion outbreak in 1943. Indeed, the records show that metritis is a wholly inconstant complication.

As for the agglutination test, several fairly plain concepts can be constructed from the records. Firstly the periods during which infected goats remained blood-positive have been very short; e.g. of 53 goats, 28 reacted on one occasion, 15 on two occasions, 10 on three and 0 on four occasions, when

*Many of the records used here are due to the work (unpublished) of M. Abdussalam, L.V.P. who carefully investigated the problem in 1936; his kindness in permitting their use is gratefully acknowledged.

tested four or more times at very irregular intervals averaging about six months apart. Indeed, the inspection of individual records suggests that few goats were blood-positive for more than two months at a time. Secondly, the appearance of positive blood reactions in relation to abortions was very irregular (Table XLVII).

TABLE XLVII

Blood reactions in relation to abortions

Before abortion				Months	After abortion			
−12	−9	−6	−3	0	+3	+6	+9	+12
1	2	0	0	4	2	1	2	1

13 Observations.

Figures= numbers of + reactors found at the month shown. Six of the goats reacting at times distant from abortion were negative to tests occurring nearer to abortion. The remainder were not tested in between.

Thirdly, of 66 goats that aborted between 1929 and 1935, 37 were blood-tested. Of these, 17 were positive, four were doubtful and 16 were negative. Now an inspection of Table XLVIII shows that amongst these 17 reactors, 12 (70 per cent) required four or more tests for their detection and, in view, of this, it is fair to assume that some 70 per cent of the 11 negative goats that were tested not oftner than twice, would be positive if tested four times or oftner. The final picture of the test results of those 37 aborting goats then, appears as in Table (XLIX).

TABLE XLVIII

The final picture of test results

Positive reaction occurs	6 tests (or more)	5 tests	4 tests	3 tests	2 tests	1 test
Never	3	1	1	0	2	9
Once	4	2	1	1	1	3
Twice	4	0	0	0	0	0
Thrice (or oftner)	1	0	0	0	0	0

Figures in Table= No. of goats reacting as shown in column 1 when tested the No. of times shown at c olumn heads.

TABLE XLIX

Number of tests necessary

Reaction	Actual	Per cent
Positive	17 to 25	46 to 65
Doubtful	4	10
Negative	16 to 8	43 to 21

37 Observations.

Further, the probability is that the higher positive figures are correct, suggesting that Brucellosis caused the majority of abortions.

It must, however, be admitted that many of these goats became positive as late as several months after abortion and it can be supposed that they were infected during the interval. All that transpires

then from the earlier investigations is that Brucellosis is present in the herd, although its very irregular manifestations in the form of agglutination responses leave much doubt as to whether it was the sole cause of the abortions.

The history after 1935 is very similar. As a result of the bad abortion years of 1934, 1935 and 1937 special pains were taken from 1937 onwards in the testing and disposal of reacting goats.* The abortions diminished until in 1939 there were none; whereupon the testing regulations were relaxed. In February 1940, the whole herd was tested and found practically disease-free, but during the 10 succeeding months 11 (4·4 per cent) goats aborted, while in 1942 and 1943 the abortion rate had again become serious. In May 1943, the whole herd was tested, 10 per cent being positive; while in July of the same year reactors had increased to 22 per cent. Of 15 animals aborting up to this time, rather less than 50 per cent were positive, as also were 6 per cent of the young stock.

The 1943 outbreak was carefully investigated by one of the writer's staff. Unfortunately, as with all previous investigators, he arrived after the abortions had ceased. However, cultures (tryptose broth, MacConkey's and Robertson's media) and inoculations (guinea-pig) were made of the cream and vaginal secretions of the recently aborted animals. Vaginal mucus was also examined for protozoan infection. From none of these preparations could any pathogen of known significance, including *Brucella*, be isolated. The most commonly occurring commensals were gram-positive cocci while from two cultures non-pathogenic bipolar organisms were isolated. A Listerella-like organism which has been described on page 184 was secured from one other culture.

The whole history of this endemic is unsatisfactory, mainly on account of the irregularities of the agglutination test and failures of isolation. It is quite clear, however, that a proportion of goats have been infected with Brucellosis and it may be supposed that many abortions have been due to this infection. The supposition is supported by the fluctuations in the abortion rate following the tightening and relaxation of the test-and-disposal orders. Plainly, Brucellosis should be permanently eradicated from the herd, a process not without difficulty, as new goats are constantly being purchased and existing goats must occasionally be exposed to infected cattle. If after permanent eradication abortions continue, then plainly, there is room for a further investigation which should begin, at least, by a following up of the investigation of the Listerella-like organism already described. It is to be noted that rainfall, intercurrent extra-genital disease, diet deficiencies, and food-poisoning cannot be implicated in this endemic.

The following table gives the various sections of the final report which correspond with different items of the technical programme.

Item of the technical programme	Section or sections of the final report
1	I, II and III
2	IV
3	V
4	VI
5	VII

Sections VIII and IX of the final report deal with relevant matters not clearly defined in the technical programme.

SUMMARY

This report is a detailed record of a five year's scheme of investigation into abortion amongst the cattle, sheep and goats of India. The text is divided into nine main sections, all, but the two last, being devoted to the subject of Brucellosis.

* In 1930-31 on the orders of the Director of Veterinary Services of the Province reacting goats were disposed of, but before 1937 it is very difficult to trace how long this order was rigorously enacted.

Sections I, II and III. Epidemiology

Section I outlines the distribution of Brucellosis in India, and at the sale time distinguishes two forms of the disease viz., an imported and an indigenous. The former occurs in organized farms whilst the latter is shown to have a remarkable incidence in its being almost entirely confined to the villages of the humid Indian peninsula. The character of indigenous strains is peculiar. Brucellosis of any sort was found to be rare in the arid north-west and fairly common in the south. Details are given of both farm and village outbreaks.

Section II is mainly composed of epidemiological data for the various classes of Indian stock (e.g., mean figures of frequency of abortion). Examples of exceptional occurrences are also given. It is concluded that the descending order of the susceptibility amongst the various stock is European cows; crossbred European-zebu; indigenous zebu; buffaloes; goats; sheep.

Section III reviews, in the light of the field findings, the probable means by which Brucellosis is spread in India, and it appears that elementary hygiene and climate are of supreme importance in this respect. The extremes of the Indian climate offer an unusual opportunity for the study of the effect of climate on the spread of the disease and a hypothesis that moisture is an almost essential factor in the dissemination of Brucellosis is deduced from the distribution of the infection in this country. This hypothesis not only fits the observed facts but is supported by an experimental demonstration that *Brucella* die much more rapidly in dry, than in moist, air.

Section IV. Bacteriology

Section IV records the examination of some 50 Indian *Brucella* strains. From the aspect of virulence (white mice tests), antigenic structure and sensitiveness and tendency to dissociate these strains are unremarkable. In one respect only do they differ from those of Europe, i.e., some 40 per cent of all Indian field strains have typing characteristics intermediate between *abortus* and *melitensis*. All village strains were of this nature and evidence is adduced which suggests this is an indigenous strain.

Section V. Diagnosis

The standards for an all-India tube agglutination test are discussed and fixed in section V. This test is intended for fine diagnosis but the use of the quick test is urged for survey work. The reliability and specificity of these tests in Indian animals are reviewed and examples of the interpretation of field results are given. A number of optimum proportion tests fixing the best density for tube antigen are reported and a simple method used for standardizing the density against reconstructed dried serum is explained.

Section VI. Human infection

Section VI discusses the human aspect of Brucellosis in India. Reports of the prevalence of melitensis infection in this country, published during the first decade of the century, are reconciled with contemporary reports, asserting the scarcity of the disease by supposing that spread of infection from the Mediterranean basin is obstructed by the interposing deserts, and that the infection referred to in the earlier records was introduced into strictly localized areas by trooping movements from Malta. Man's liability to infection by the indigenous strains of south India is discussed, but little real evidence can be adduced in this connection.

Section VII. Control

Section VII deals with control of Brucellosis (*a*) by hygiene and (*b*) by vaccination. In India, it is not usually feasible to control minor endemics by sufficiently frequent tests followed by disposal. It has been noticed, however, that where zebu live more or less on the ranch system, in dry sunny climate, Brucellosis is almost self-eliminating; this tendency, augmented by a brief maternal segrega-

tion during the infections calving periods is used as the basis of a system of control. It appears that the method may be of value in the semi-desert areas of India, at least.

The subsection on vaccination first records an examination of the behaviour of overseas vaccine strains under Indian conditions. The bad keeping qualities of the vaccine are noted, and by means of plate counts, the effects of extreme temperature variations in temperatures, and shaking on the mortality of *Brucella* suspensions is investigated. Temperatures of upwards of 80°F. were found to cause rapid death, variations of temperature had no obvious lethal effect, whilst shaking is undoubtedly the cause of a certain mortality. By means of several counting experiments, a substrate was evolved for the vaccine that materially improved its keeping qualities in extreme conditions. Recommendations follow regarding the preparation, issue and application of the vaccine in the field. The section concludes with a few notes on the early results of calfhood and adult vaccination in Indian farms.

Section VIII. Other infectious abortion

In this section, attention is briefly called to infectious abortion other than Brucellosis. The apparent absence of *T. foetus* from India is noted, as also the rareness of other abortifacient infections such as Listerellosis. A new bacterium is described that may in some measure be concerned with goat abortion.

Section IX. Non-specific abortion

Circumstances commonly leading to non-specific abortion are discussed in section IX.. In the zebu, such occurrences are thought to be rare and almost exclusively associated with systemic disturbances such as fevers. To a much greater degree this also applies to the buffalo, in which animal obscure epidemics of abortion are frequently noted, often associable with extreme rainfall. This phenomenon is discussed at some length.

Both sheep and goats are very liable to abort as the result of systemic shocks and it is not improbable that the organism mentioned in section VIII, or some other undescribed microbe is the cause of certain obscure epizootic abortions amongst goats in the Punjab.

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MGIPC—M—III-1-9—11-2-49—750.

Original Articles

IMPROVEMENT OF WOOL QUALITY BY SELECTIVE BREEDING IN BIKANERI AND LOHI SHEEP

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(Received for publication on 9 July 1948)

QUALITY in wool is a vague term. It is intended to indicate a combination of certain characters like medullation, length, fineness, strength and elasticity etc., which help to improve the spinning quality of the fibres ultimately affecting the merits of the finished cloth. Most of our Indian breeds of sheep produce only the carpet class of wool i.e., a mixture of varying proportions of wool and hair. The presence of hair (technically known as medullated fibres) is a major defect in wool as it prevents its use for the manufacture of good clothing material in comparison to Merino and Cross bred wools which are either totally free from medullation or show only a slight amount of hairiness. The presence of hair in wool reduces its spinning quality, gives roughness to the cloth, is weaker in tensile strength and does not take up the dyes. Any attempt to improve the quality of Indian wool must primarily include the elimination or reduction of hair in the fleece. Medullation in wool has been found to be highly correlated to fineness, strength and handle of wool, thereby indicating that improvement in medullation alone will greatly affect the quality of Indian wools.

With the partition of the country, the problem of making the Indian Union self-sufficient as regards its requirements of various types of wool has assumed greater importance. To achieve the object in view, the Indian Council of Agricultural Research, has recently drawn up a plan for the production of various types of wool, both by grading up with the Merino and by selective breeding of some of the indigenous breeds. The plan for selective breeding is based on the work done at Hissar.

While going through a large number of fleece samples of Bikaneri and Lohi sheep in the wool analysis laboratory at the Hissar Farm, it was noticed that there was wide variation, ranging from a purely woolly fleece to a totally hairy type. It was, therefore, felt that the evolution of a clothing type of sheep should not be impossible. Accordingly, the improvement of fleece quality by selective breeding was started in both the breeds in the year 1943-44. In the course of about five years, it has been noticed that with the help of an improved system of judging the fleece quality and resorting to judicious selection, an appreciable improvement in the fleece quality of the flocks can be affected.

Testing of fleeces and fixing of quality standards

As a pre-requisite to improving the quality of wool, an accurate method for the testing of individual fleeces and laying down some quality standards was considered essential. It had already been experienced that assessment of fleece quality by feel was an unreliable method and often led to incorrect results. Some standard laboratory method was, therefore, necessary to fulfil this requirement. This was accomplished by adopting the 'rapid benzol test' described by Nanda, Singh and Mongre [1946]. Small representative samples were taken from the shoulder region of each sheep and tested in the wool laboratory for the percentage of medullation in the fleece. This method afforded satisfactory results for the purpose in view.

Quality standards of foreign wools are well known in the trade, but India produces mostly coarse type of wool for which there are no suitable standards. Any type of coarse or fine wool, irrespective of its utility value in the manufacturing process, is classed as a carpet wool. Certain arbitrary standards were laid down for use in the present work. To fix up these working standards, a large number of Bikaneri and Lohi sheep were first surveyed for the quality of wool on medullation basis, and it was

noticed that in these breeds, the range of medullation varied from 0 to 100 per cent. On the basis of medullation of wool, fleeces were divided into the following four classes [Nanda, 1947].

A Class . . Fine woolly fleeces with 0 to 20 per cent medullation, quite suitable for the manufacture of clothing material.

B Class . . Ideal carpet type fleeces with 20 to 40 per cent medullation, best suited for carpets.

C Class . . Coarse hairy fleece with 40 to 70 per cent medullation, conforming to inferior type of carpet wool suited for low grade carpets.

D Class . . Very coarse hairy fleeces with 70 per cent and higher medullation, hardly suitable for spinning and weaving processes.

Selective breeding and inter-breeding of different classes

The procedure adopted for selective breeding consisted in classifying flocks of Bikaneri and Lohi ewes into A, B, C and D types according to the standard mentioned above. To start with, only a small percentage of better type fleeces were available, as the majority of ewes came in C and D classes. More of A and B type rams were introduced each year and the C and D type ewes were eliminated in successive years. Shoulder samples of all the male progeny at the lamb's clip and of the female progeny at the 2nd clip were tested to judge the improvement and to help in selection for future breeding. This practice, exhibited a gradual but distinct improvement in the new progeny with increased number of A and B type lambs each year. The following figures indicate the results obtained in Table I.

TABLE I

Bikaneri sheep—flock strength—500 breeding ewes

Class of progeny tested	Year during which tested	Total No. of rams used	No. of rams with A & B fleeces		No. of progeny tested	Percentage of fleeces in different classes with approximate amount of medullation			
						A	B	C	D
			A	B		0-20 per cent	20-40 per cent	40-70 per cent	70 per cent and higher
Males	1943-44	15	..	..	107	4·7	10·8	35·5	49·0
	1944-45	15	2	1	165	6·1	19·4	46·6	27·9
	1945-46	15	6	2	165	8·2	22·8	49·7	19·3
	1946-47	15	6	4	132	24·2	31·1	38·6	6·1
Females	1943-44	15	..	..	109	7·3	22·0	37·5	33·2
	1944-45	15	2	1	145	13·1	18·6	40·7	27·6
	1945-46	15	6	2	144	14·8	17·6	40·5	27·1
	1946-47	15	6	4	177	36·7	26·5	21·3	15·5
Lohi sheep—flock strength—200 breeding ewes									
Males	1943-44	6	..	..	93	11·8	11·8	40·8	35·6
	1944-45	6	1	1	82	18·2	15·9	31·8	34·1
	1945-46	7	3	1	84	24·4	15·1	40·7	19·8
	1946-47	6	4	2	63	31·7	23·8	36·5	8·0
	1947-48	6	5	1	94	42·5	23·4	34·1	..
Females	1943-44	6	..	..	51	7·8	13·7	39·2	39·2
	1944-45	6	1	1	58	12·0	10·3	44·8	32·8
	1945-46	7	3	1	74	20·2	18·9	35·2	25·7
	1946-47	6	4	2	87	31·0	24·1	40·2	4·7
	1947-48	6	5	1	86	38·8	23·6	37·6	..

It is evident from the above Table I that in about 4 to 5 years' time, the percentage of progeny with finer type of wool increased from 6·0 to 30·4 per cent in the Bikaneri flock and from 9·8 to 40·6 per cent in Lohi flock with a decrease in the coarse and hairy types from 41·1 to 10·8 per cent and 37·4 per cent to 0 per cent in the two flocks respectively. With continued selection, complete elimination of C and D types and the establishment of pure A or B type flock is now envisaged. Improvement would have been quicker if it had been possible to use all A class rams from the very first year. This was not possible as only a limited number of male lambs upto the required standard were available for selection in the beginning. The comparatively quicker improvement in the Lohi flock was due to the larger number of A and B type rams used.

With a view to study the results of inter-breeding among different classes, the inheritance of medullation in the grading up of C and D type ewes with A and B type rams and the possibility of evolving a clothing type woolly sheep by inter-crossing of A class sheep, breeding records of different crosses during the last three to four years have been analyzed. The information, thus obtained, provides a basis for formulating a general plan for the improvement of fleece quality of Indian sheep. The results obtained by various crosses are shown in Table II.

Table II

Bikaneri sheep

Type of rams	Type of ewes crossed	No. of ewes bred	Percentage of progeny in different classes			
			A	B	C	D
Unclassed	Unclassed	209	15·3	22·5	45·4	16·8
	A	4	25·0	50·0	25·0	..
	B	25	20·0	36·0	32·0	12·0
	C	49	18·3	28·5	42·8	10·4
	D	23	12·6	21·7	56·5	9·2
A	Unclassed	121	33·9	31·4	25·6	9·1
	A	26	61·5	34·6	3·9	..
	B	31	51·6	22·6	25·8	..
	C	49	32·6	44·8	20·4	2·2
	D	25	26·4	32·3	35·3	6·0
B	Unclassed	79	8·8	32·9	45·5	12·8
	A	3	66·6	..	33·3	..
	B	12	33·3	33·4	33·3	..
	C	25	16·0	48·0	20·0	16·0
	D	14	7·1	35·7	35·7	11·5
C	Unclassed	72	4·2	6·9	45·8	43·1
	A	1	..	..	100·0	..
	B	2	50·0	..	50·0	..
	C	5	..	20·0	40·0	40·0

TABLE II—*contd.*

Lohi sheep

Type of rams	Type of ewes crossed	No. of ewes bred	Percentage of progeny in different classes			
			A	B	C	D
Unclassed						
	C	42	16·0	12·0	40·0	32·0
	D	26	3·2	6·2	50·0	40·6
A	A	60	73·1	20·9	6·0	..
	B	22	40·9	22·7	36·4	..
	C	145	32·5	24·2	40·7	2·6
	D	72	15·5	29·7	46·4	8·4
B	B	42	43·7	29·2	27·1	..
	C	46	12·3	21·0	47·4	19·3
	D	1	..	..	100·0	..
C	B	12	18·8	..	50·0	31·2
	C	15	11·1	5·5	55·5	27·7
	D	16	15·8	10·5	31·5	42·2

The following conclusions can be drawn from the above Table :—

(i) Inter-breeding of A type sheep produces a high per cent of A type of progeny.

(ii) There is a gradual increase in the A and B class progeny by the use of A class rams as the percentage of medullation decreases in the ewes from D to A types.

(iii) Percentage of medullation in the progeny is directly proportional to the percentage of medullation in the ewes or the rams.

(iv) Grading up of C and D type ewes with A type rams is possible but the process is slow.

It is thus apparent that for quick improvement, A type rams should be included in the flocks at the earliest opportunity and C and D type ewes should be eliminated. B type rams should not be used when A type rams are available.

During the course of above work, it was noticed that with the increase of wool type fleeces, there was some deterioration in the size, live-weight and stamina of the animals. The sheep with finer wool appeared to be more delicate and lacked bone. The Table III indicates the actual performance of the four types of sheep in the Bikaneri flock.

TABLE III

Flock	Class of ewes	No. of ewes	Average wool yield in 2 clips per year		Average body measurements in inches after first shearing			Per cent Fertility	Average weight of ewes in lb.		Average weight of lambs in lb.		Per cent mortality	
					Girth	Length	Ht.		When put to rams	Just after lambing	At birth	At wng.	In lambs upto weaning	In ewes during the year
			lb.	oz.										
A	A	43	4	12·0	28·7	22·0	24·9	69·7	60·2	64·7	5·8	32·3	26·6	4·6
	B	56	4	15·3	29·5	22·6	25·5	76·8	63·5	68·1	5·8	35·2	11·9	5·3
	C	91	5	0·4	30·2	22·7	25·7	70·3	67·3	68·3	5·7	33·7	13·4	9·9
	D	91	4	11·1	30·3	22·8	25·9	70·3	66·8	66·4	5·6	34·3	18·7	11·0
B	A	18	5	12·4	..	..	..	66·6	62·7	61·7	5·4	28·7	..	5·5
	B	25	5	5·8	..	..	..	87·5	68·0	66·0	5·8	34·5	4·0	10·0
	C	45	5	14·7	..	..	..	74·4	68·8	67·1	6·0	36·8	9·4	4·4
	D	48	5	10·4	..	..	..	71·4	72·1	70·0	5·7	35·7	23·4	22·0

(Body measurements in the A flock are based on a representative sample of 148 ewes including all classes.)

Though the differences in the performance of different classes of ewes in both the flocks are not very significant, the under noted inferences are however apparent :

(i) 'A' type is definitely a lighter and a smaller sheep.
(ii) Fertility in the A class sheep is lower as compared with other classes.
(iii) Although the birth weights of the lambs from different classes are uniform, yet the weaning weights of lambs from A class ewes are the lowest. This indirectly indicates a poor flow of milk in these ewes.
(iv) Inspite of the fact that A class is a lighter sheep, there are no significant differences in the average wool yields during the year.
(v) Except for a slightly heavier body weight, D class ewes possess little advantage over other classes. It is therefore useless to retain these hairy type of ewes in the flock.

Besides the above inferences, a slightly higher mortality in the A class lambs is also apparent. As the year under study was an abnormal one, with heavy rains and severe adverse conditions, this factor will have to be studied during normal years.

With the improvement in fleece quality, another feature observed was a remarkable decrease in the percentage of twin births in Lohi sheep. This breed is well- known for its milk capacity and a high percentage of twins. The percentage of twins from different classes of ewes from the year 1943 to 1946 were as below :

Class of ewes	Percentage of twin births
A	12·5
B	15·9
C	18·4
D	20·0

PROGENY TEST OF RAMS

Progeny test is a reliable method to ascertain the transmitting qualities of a sire and is one of the surest means to bring about a substantial improvement in any class of livestock. While, carrying out selective breeding for fleece quality, it was noticed that although A class rams always produced

higher percentage of better class progeny when used on different classes of ewes, their transmitting ability was highly variable when compared with one another. This fact is revealed from Table IV below :

TABLE IV

Progeny test of rams

No. of ram	Class	Class of ewes bred	Number of lambs dropped	Percentage of lambs in different classes				Remarks
				A	B	C	D	
314	A	A	9	33·0	66·7	..	..	*Nil*
400	A	A	58	79·3	13·8	6·9	..	
431	A	C	38	23·7	15·8	55·2	5·3	
364	A	C	30	40·0	30·0	23·3	6·7	
637	A	C	32	28·1	43·8	28·1	..	
612	A	C	31	45·1	12·9	42·0	..	
314	A	D	24	25·0	50·0	25·0	..	
518	A	D	30	16·6	40·0	40·0	3·4	
637	A	D	6	33·3	33·3	33·4	..	
624	A	D	6	33·3	33·3	33·4	..	
612	A	D	6	16·6	..	83·4	..	

The cause for this variable prepotency in the rams can either be attributed to variable inheritance or variation in the medullation percentage of the fleece which ranges from 0 to 20 per cent in the shoulder samples. On analysing the whole fleece, significant variation of quality has been observed in different regions of the sheep's body. This fact further widens the medullation range in different individuals when it is taken collectively for the whole fleece. In order to obtain the maximum advantage in the selective breeding, the following points should be borne in mind :

(i) All rams should invariably be subjected to the progeny test at the close of the breeding season and only those rams should be retained for breeding which show a high percentage of A class progeny.

(ii) Only those rams should be used for breeding which show the least percentage of medullation.

(iii) While assessing the quality of a ram, medullation percentage should not be relied upon on shoulder sample alone but the collective result of the whole fleece should be evaluated by testing representative samples from different regions of the body.

DISCUSSION

It is evident from the above that improvement of wool quality in the Bikaneri and Lohi sheep is possible under careful selective breeding. Large scale improvement in the village flocks can be undertaken, on the same lines, by establishing of breeding farms and issuing of tested rams in different localities. Before any large-scale plans are taken up throughout the country certain important aspects deserve special attention.

Firstly, a general survey of the sheep breeds throughout the country is essential to ascertain their potentialities for the improvement of wool quality in relation to climate and other environmental factors. India as a whole, presents a wide range in its sheep breeds reared under a variety of conditions. From the South to the North, there is a gradual improvement in the quality of sheep

till the best breeds are found in the northern parts of the country. The range of climate over this area is also variable to the same extent. This shows that limitations of climate etc., are probably responsible for the inherent qualities of different breeds. Drastic changes under such circumstances may not produce appreciable results. Two alternatives are, therefore, suggested, before any definite lines are laid down for improvement. In those breeds which are extremely hairy in the fleece and possess little potentialities for a woolly type, attempts to improve their character to a clothing type must be tried with caution. A suitable plan would be to bring these up to an ideal carpet wool producing sheep, corresponding with B type animals described in this note. For dealing with other breeds, which are located in favourable environments, efforts may be made to breed them for a clothing type sheep.

Secondly, much of the impetus to produce a better class wool depends upon the price obtained in the market. Unfortunately, market conditions are very unsatisfactory in the country and there is no encouragement for the breeders of better quality wool. All wools whether good or poor, classed or unclassed and washed or unwashed sell practically at the same price in villages. To encourage breeders to produce better wool, it is essential that the markets should be organized on systematic lines and the prices controlled on some recognized standard basis.

Thirdly, it has been observed that the fine quality wool has a limited use as a cottage industry. This is because no head-way has been made in providing suitable hand looms for treating this fibre. With the increase in production of good quality wool, it is necessary that particular attention should be paid to this item. In order to encourage the production of a better quality wool, adequate safeguards will also be needed to restrict imports of foreign wool.

SUMMARY

1. A method for improving wool quality in the Bikaneri and Lohi sheep by selective breeding has been suggested.

2. Workable standards of wool quality according to their utility value in the manufacturing processes have been laid down on medullation basis.

3. Breeding from A type rams, higher percentage of better class progeny is obtained. The percentage of medullation in the fleeces of the progeny appears to be directly related to the percentage of medullation in the ewes and rams.

4. Inter-breeding of A type sheep (having less than 20 per cent medullation) produces a high percentage of better class progeny, indicating definite possibilities for evolving a clothing type sheep from both these breeds.

5. Improvement in fleece quality appears to result in a decrease in the size, live weight, fertility of ewes and growth of lambs. It is also apparent that these deficiencies can be made good by fixing minimum standards for rams and paying special attention to the milk flow of the dams at the time of selection.

6. It has been observed that the A class rams possess variable prepotency in transmitting their characters to the progeny. This may be due to a variable percentage of medullation in their fleece which range from 0 to 20 per cent. To ensure maximum improvement in the flock, all rams should be subjected to the progeny test at the close of the breeding season and only desirable rams should be retained for breeding. In addition, assessing of wool quality of the ram should be based on testing representative samples from the different regions of the fleece and not from the shoulder alone.

7. For large-scale improvement in the village flocks, it seems, that a preliminary survey of all the sheep breeds throughout the country is necessary to lay down a definite plan. Proper control and organization of wool markets with the establishment of utilization centres to deal with the fine quality wool in hand looms are additional requirements to encourage breeders to produce fine quality of wool.

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HAY-BOX AND SIMMERING PROCESSES OF PRESERVING MILK—THEIR EFFECT ON GHEE PRODUCTION

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(Received for publication on 5 June 1948)

(With one text figure)

MAJOR portion of *ghee* marketed in India comes from the villages. The quality of the product and the economics of the *ghee* trade are linked closely with the regional rural practices in *ghee* making. Since *ghee* is mainly obtained from surplus milk and as the holdings of the villager are small, the morning and evening milk are generally pooled together for making measurable quantity of *ghee*. In the Punjab villages, the usual practice is to give a form of heat treatment to the morning milk so that it may remain sweet until the evening's surplus is added preparatory to curdling and separation of butterfat. The form of heat treatment adopted by the villagers is to bring the morning milk to boil and maintain it simmering the whole day. Cowdung cake is used as fuel which not only entails the wastage of a valuable manure but the smoky flame it gives in an improperly designed village hearth often contributes towards undesirable flavour in *ghee* and the by-product *lassi*.

The 'hay-box method of preserving milk hot' as recommended by Read [1937] obviates the defects observed in the village simmering process. The hay-box is a wooden box, the length and breadth of which is about twice the greatest diameter of the milk pot and about ten inches more than its height. The box is packed firmly, but not too tightly with *bhusa*, leaving just enough space in the centre of the box for holding the milk vessel which may be a narrow-necked round-bottomed earthenware *goli*. The operational part in the use of the hay-box consists in bringing the morning milk, held in the earthen pot, to boil and as soon as possible the pot with its content is transferred in the hay-box. More *bhusa* is then packed into the box all round the pot up to the edge of its neck. The mouth of the pot is then closed with a wooden cap grooved inside the peripheral round so that it can sit tightly over the rim of the pot and a layer of *bhusa* can be spread on top of it. After closing the lid, the box is put out of the way until the evening. The thermal insulation secured by this method preserves the milk fairly hot so that it can easily be transferred into the vessel containing the evening milk for mixing and subsequent curdling.

As against continuous simmering, the hay-box method of preservation is undoubtedly capable of bringing about considerable fuel economy. Read, however, claims that this method of preservation effects a greater outturn in *ghee* by larger recovery of milkfat and also produces a better quality *ghee* and *lassi*. In view of the importance of this claim, the Indian Council of Agricultural Research had sponsored a scheme of investigation to ascertain the comparative merit of the hay-box method against the customary simmering process. In the present communication are reported the results of the experiments carried out at two centres, *viz.*, Indian Dairy Research Institute, Bangalore and Co-operative *ghee* Producers' Union, Jhallar, in the Central Provinces to throw light on the following aspects :

(*a*) The relative merit of the methods of treatment on the preservation of milk.

(*b*) The effect of the methods of preservation on (i) the outturn of *ghee*, (ii) the market quality of freshly prepared *ghee* and (iii) the storage life of *ghee*.

To include seasonal variation in the quality of milk the experiment at Bangalore was conducted in (i) July-August (early monsoon), (ii) October-November (late monsoon) and (iii) December-January (winter) and at Jhallar in rainy and winter seasons.

Experimental

The studies on the preservation of milk and subsequent preparation of *ghee* by simmering and hay-box methods were carried out by using two sets of processing conditions. In the first set, processing of raw milk consisted in bringing it just to boil. In the second set, boiling was continued for 10 minutes.

In the hay-box method two sets of duplicate lots of 7½ lb. morning's raw milk filled in well seasoned mud pots of 8 lb. capacity were given initial treatmnets as mentioned above. Immediately after the treatment the pots were transferred into uniformly packed hay-boxes and left undisturbed till evening. In the evening, warm milk in the pot kept in hay-box was transferred into a second pot of 50 lb. capacity containing 7½ lb. raw evening milk. Morning's warm preserved milk and evening's cold raw milk together with 2 per cent (Bangalore) or 1½ per cent (Jhallar) of lactic starter from stock supply were thoroughly mixed and allowed to ripen.

In the simmering method, the two sets of duplicate lots of 7½ lb. morning's raw milk filled in seasoned mud pots of 50 lb. capacity were given the prescribed heat treatment. The pots with their contents were then transferred to separate *choolas* with smouldering fire of cowdung cakes. The milk in the pot was thus kept simmering until evening when 7½ lb. of evening's raw milk was added. The mixed milk was then treated in the same way as in the hay-box method.

The treated milk samples by different methods were allowed to ripen for a period of 15-16 hours under atmospheric conditions. The curd at the end of the ripening period was thoroughly mixed and samples were taken for determining the acidity before churning. Churning was done in the same vessel with the aid of indigenous churning device. No special precaution was taken in controlling the temperature of churning except that cold water was added from time to time. The total quantity of cold water added was equivalent to that of the curd. The churning was discontinued when the butter granules were formed. After allowing 15 minutes for settling, the butter granules were collected and worked up to a lump as practised in the villages. As soon as a sample of butter was prepared, it was tested for acidity, curd content and moisture. The quality of butter-milk was also examined for its taste and flavour.

Soon after butter from each treatment was prepared, it was clarified into *ghee* in aluminium *degchies* held over kerosene stove. During clarification the melt was continuously stirred and the flame of the stove was so adjusted as to finish the removal of moisture between 110-120°C. After allowing to cool, the *ghee* samples were strained through fine muslin cloth and their weight recorded. The fresh *ghee* samples under each treatment made in different seasons were judged for their market quality according to the following basis :

Points for judging	Score allotted
I. Aroma	30
II. Flavour	30
III. Texture	30
IV. Colour	10
Total	100

The fresh *ghee* samples were also chemically examined for Saponification Value (S. V.), Reichert Meissl Value (R.M.), Polenske Value (P. V.), Iodine Value (I. V.), Peroxide Value (Per. V) and Acidity (per cent oleic). The methods followed in the determination of these constants were the same as given in the Miscellaneous Bulletin No. 64 of the Indian Council of Agricultural Research. The keeping quality of the samples was followed by determining the peroxide value at regular intervals of representative preparations which were filled in 1 lb. tins of uniform size and stored at a constant temperature of 104°F. The market quality and the physico-chemical constants of the stored samples were also judged at the end of the storage period of three months.

In view of the fact that milk used at Bangalore was mixed from cow and buffalo, and at Jahallar from buffalo only, *ghee* was also prepared from the same aliquots of milk by the standard cream-butter method to facilitate comparison of yield, market quality and storage property of *ghee* obtained by the methods under investigation.

RESULTS AND DISCUSSION

The effect of the method of heat treatment and subsequent preservation on the quality milk, curd, lassi and butter : The role of the hay-box is essentially to offer insulation to heat processed milk so that at an elevated temperature it can remain sweet till the evening's collection is ready to be added. The efficiency of the method is to be judged, therefore, from (i) the degree of heat conservation it affords against continuous heat transmission entailed in the simmering of milk and (ii) the extent of proliferation of the surviving micro-organisms.

The temperature of hay-box preserved and simmered milk just before evening quota is added has been found to be 120-130°F and 145-155°F respectively. The lower and upper limits of the range are obviously influenced by the seasonal atmospheric temperature.

In Table I are given the average figures of acidity, total count and presumptive coliform in the morning milk at different stages of its handling.

TABLE I

Quality of morning milk at different stages

Particulars	Per cent acidity (lactic)	Total count	Presumptive coliform
1. Raw	0·15	1,000,000—4,000,000	+ve (1 : 1000)
2. Processed (just boiled)	0·16	600—1,200	—ve (1 : 10)
3. Processed (boiled for 10 min.)	0·16	200—1,000	—ve (1 : 10)
4. Preserved hay-box (just boiled)	0·22	10,000—12,000	—ve (1 : 10)
5. Preserved simmering (just boiled)	0·18	8,000—10,000	—ve (1 : 10)
6. Preserved hay box (boiled for 10 min.)	0·22	8,000—10,000	—ve (1 : 10)
7. Preserved simmering (boiled for 10 min.)	0·18	5,000—8,000	—ve (1 : 10)

A perusal of the data shows that the initial processing by maintaining the boiling temperature for 10 minutes is relatively more efficient than just bringing it to boil as is evident from the lower bacterial count at different stages, both in hay-box and simmering method of preservation.

Although the total bacterial count at the end of the preservation is about the same for the two methods, the increase in acidity by 46·7 per cent in hay-box as compared to 20·0 per cent in simmering suggests that in the former method of preservation, there is relatively much greater proliferation in the acid-forming thermoduric organism which cannot be counted by plating at 37°C.

The use of hay-box as a means to preserve milk has been doubted by the Agricultural Bacteriologist, Punjab Agricultural College, Lyallpur. In a private communication, he reports that milk samples preserved in hay-box in hot weather show very poor keeping quality and are often found curdled before the evening milk can be added. The deterioration is due to the growth of micro-organism active at high temperatures. In winter, although the temperature of milk stored in hay-box is fairly high, the curdling seldom takes place before the evening milk is ready to be added. This is owing to the low initial bacterial contamination of milk usually observed during this season and

shorter day, or, the period of storage. In the course of the present investigation, neither at Bangalore nor at Jhallar there was any instance of the actual curdling of the morning milk. Perhaps this might have been due to the shorter interval (6-7 hours) of preservation. Experimental data obtained recently in the Dairy Technology Section of the Indian Dairy Research Institute, Bangalore, show that if milk is collected in hot and humid season and stored at an elevated temperature varying from 120-140°F., curdling of milk takes place within 8 hours. As in actual practice, the preservation period in the villages may have to be as long as 12 hours, the hay-box method seems to be of doubtful utility.

Srinivasan and Banerjee [1946] and Rangappa *et al* [1946], have recently shown that more than the quantitative, the qualitative distribution of microflora in milk, prior to its ripening, decides the nature of fermentation and thereby the quality of the curd on which, in turn, will depend the quality of butter and butter-milk. In the present methods where the evening's raw milk is added to morning's processed milk, the significance of initial processing on the quality of the curd or butter or butter-milk is practically lost because it has been observed that the addition of evening's raw milk invariably introduces coliform contamination which is absent in morning's processed and preserved milk. The re-entrance of coliform contamination is indicative of the presence of harmful organism which would eventually frustrate the use of lactic starter in setting up the desired curd. This is borne out by the fact that the curd in all the methods has been slightly blown up, and possessed of ropy texture, with the rising up of the curd separately from the whey. Due to this uncertain course of fermentation, irrespective of the method, butter-milk (*lassi*) obtained at Bangalore was uniformly of inferior quality. According to the opinion of Jhallar workers butter-milk from simmering process was slightly more palatable than that produced by the hay-box method. In Table II are given the range of acidity in curd and butter as also of moisture and curd content in butter.

TABLE II

Range of acidity in curd and butter and moisture and curd content in butter

Method	Acidity (per cent lactic)				Moisture per cent in butter		Curd per cent in butter	
	Curd		Butter					
	Bangalore	Jhallar	Bangalore	Jhallar	Bangalore	Jhallar	Bangalore	Jhallar
1. Hay-box (just boiled) .	0·70 to 0·85	0·85 to 0·86	0·20 to 0·27	0·21 to 0·22	19·5 to 20·7	21·8 to 22·2	1·9 to 2·1	1·2 to 1·6
2. Simmering (just boiled)	0·80 to 0·85	0·81 to 0·86	0·22 to 0·25	0·22 to 0·24	19·6 to 25·0	21·9 to 22·1	1·9 to 2·1	1·1 to 1·7
3. Hay-box (boiled for 10 min.)	0·80 to 0·85	0·86 to 0·89	0·21 to 0·31	0·21 to 0·22	19·0 to 19·5	22·3 to 23·0	1·9 to 2·2	1·1 to 1·2
4. Simmering (boiled for 10 min.)	0·75 to 0·90	0·75 to 0·86	0·22 to 0·31	0·22 to 0·24	19·0 to 20·0	21·1 to 23·6	1·9 to 2·1	1·4 to 1·6

Outturn of ghee : Notwithstanding the close liaison in the technological details of manufacture the differences in the origin of the initial raw material and other environmental factors were bound to affect the quantitative yield of *ghee* per unit weight of milk taken at the two centres. As the direct comparison of the data may prove confusing, it is considered convenient to express the relative yields by hay-box and simmering methods as the coefficient of the yield from the standard cream-butter method used both at Bangalore and Jhallar. In Table III, the data have been set out accordingly.

TABLE III

The comparative yield of ghee *by simmering and hay-box method*

No.	Method Employed	Outturn expressed as coefficient* of yield of cream-butter method		Remarks
		Bangalore	Jhallar	
1	Hay-box (just boiled) . . .	92·2	94·2	The data have been calculated from the average of 18 and 24 replications carried out at Bangalore and Jhallar respectively. The replicates were equitably distributed in the different seasons of the year.
2	Simmering (just boiled) . . .	89·6	93·2	
3	Hay-box (10 min. boiled) . . .	91·7	95·2	
4	Simmering (10 min. boiled) . .	89·5	92·4	

* For the same unit weight of milk,

$$\frac{\text{Av. } ghee \text{ yield by hay-box or simmering}}{\text{Av. } ghee \text{ yield by cream-butter method}} \times 100 = \text{Coefficient of yield}$$

Figures in Table III indicate that in either place the use of the hay-box has led to a slightly increased yield over that of the simmering process. The increased yield is about 2·4 to 2·9 per cent at Bangalore and between 1·7 to 3·0 per cent at Jhallar. The slightly lower yield by the simmering process may be an effect arising out of high temperature processing of milk for prolonged period bringing in its train extensive denaturation of milk protein. The latter in turn gets incorporated with the butter and at the time of clarification forms a relatively larger *ghee* residue absorbing more of the milk fat which would otherwise pass out in the *ghee* layer at the time of filtration.

A careful scrutiny of the data on outturn of *ghee* prepared in different months of the year showed that for all the methods tried, no significant difference was observed at Bangalore which could be ascribed to seasonal effect. Whereas at Jhallar, winter yield of *ghee* by hay-box and simmering process was about 4·6 and 5·0 per cent higher than the yield in the rainy season. The absence of seasonal influence at Bangalore may be due to equable atmospheric temperature throughout the course of the investigation.

Initial quality of freshly prepared ghee : In Table IV is set out the score and the range of variation in the physico-chemical constants of fresh *ghee* prepared by different methods.

TABLE IV

Method of processing and preservation of milk on the market quality and physico-chemical constants of fresh ghee

No.	Method	Score	S. V.	R. M.	P. V.	I. V.	Per. V	Acidity
1	Hay-box (just boiled) . .	80	223·0 to 226·0	25·5 to 26·8	1·2 to 1·4	31·7 to 32·2	0·8 to 0·9	0·21 to 0·25
2	Simmering (just boiled) .	60	224·8 to 225·8	24·9 to 26·5	1·2 to 1·3	32·8 to 33·8	0·7 to 0·9	0·23 to 0·26
3	Hay-box (10 min. boiled) .	80	224·8 to 225·8	25·5 to 26·5	1·2 to 1·3	32·3 to 33·8	0·7 to 0·9	0·21 to 0·26
4	Simmering (10 min. boiled) .	60	223·0 to 226·0	25·8 to 26·5	1·3 to 1·4	31·9 to 33·0	0·7 to 0·9	0·23 to 0·29
5	Cream butter . . .	75	222·0 to 222·8	25·5 to 26·4	1·1 to 1·2	31·0 to 32·3	0·6 to 0·9	0·16 to 0·19

It is evident from the score that, when fresh, market quality of *ghee* prepared by hay-box method is definitely superior to that of simmering method. The variation in the physico-chemical constants suggests that but for slightly higher acid value for 'hay-box' *ghee*, the method of preparation has no appreciable effect on the constants of *ghee*. There is, however, some difference in this respect between the creamery-butter and indigenous methods. In the cream-butter-*ghee*, according to the present finding, there is a slight decrease in the S. V. and P. V. indicative of the presence in *ghee* of lower proportion of the higher fatty acids and water insolnble steam volatile lower fatty acids. The development of unsaturation as evident from the I. V. and also acidity seem to be somewhat lower in the cream-butter samples. Doctor, Banerjee and Kothavalla [1940] in their study on the effect of the method of manufacture on the physico-chemical constants of *ghee* have, however, shown that the methods of preparation, such as, creamery butter and common indigenous method have no marked effect on the chemical properties of freshly made *ghee*.

Owing to the constancy in the dairy ration at Bangalore, no significant change was noticed in the initial quality of *ghee* prepared in different seasons. At Jhallar only R. M. value could be determined and it was noted that there was no marked difference in R. M. value of *ghee* prepared by different treatments. There was, however, a significant difference in this value between *ghee* samples prepared in the winter and rainy seasons. The lower value in the winter was due probably to feeding the buffaloes with cotton seed and dry fodder.

Keeping quality of stored ghee.—At the end of three months storage, the market quality of stored *ghee* samples prepared by the different methods during monsoon, post monsoon and winter seasons were judged for their market quality on the same basis of scoring as for the fresh samples. The average score, out of 100 marks, were as follows :

1. Hay-box (just boiled)	54
2. Simmering (just boiled)	44
3. Hay-box (10 min. boiled)	52
4. Simmering (10 min. boiled)	44
5. Cream-butter	70

The results of the score show that at the end of the storage period the quality of 'hay-box' *ghee* continued to remain superior to that of 'simmering' *ghee*. Compared to what was observed in the fresh samples, the range of superior marks, however, was considerably narrowed down in the stored samples. From the relative lowering in the score, it is evident that the keeping quality of 'hay-box' *ghee* is rather poorer than that of 'simmering' *ghee*; and that of either is markedly inferior to that of cream-butter-*ghee*.

In Table V are set out the results of analysis of physico-chemical constants of ghee samples after three months of storage. In Fig. 1 is presented the progressive change in the peroxide value of the *ghee* samples.

TABLE V

Physico-chemical constants of ghee *samples after three months storage at 104°F.*

No.	Method	S. V.	R. M.	P. V.	I. V.	Per. V	Acidity
	July-August (Monsoon)						
1	Hay-box (just boiled)	226·0	25·4	1·2	31·6	7·0	0·39
2	Simmering (just boiled)	226·5	24·6	1·3	34·0	5·0	0·32
3	Hay-box (boiled for 10 min.)	226·9	25·3	1·3	34·2	6·2	0·35
4	Simmering (boiled for 10 min.)	225·0	25·8	1·4	32·4	5·7	0·35
5	Creamery butter	225·3	25·5	1·3	32·3	3·7	0·26

TABLE V—*contd.*

No.	Method	S. V.	R. M.	P. V.	I. V.	Per. V	Acidity
	October-November (Post-monsoon)						
1	Hay-box (just boiled)	225·9	25·4	1·3	33·2	5·9	0·39
2	Simmering (just boiled)	225·4	25·3	1·2	33·1	4·1	0·29
3	Hay-box (boiled for 10 min.)	225·8	25·7	1·2	32·6	5·7	0·37
4	Simmering (boiled for 10 min.)	224·8	26·0	1·4	31·9	5·0	0·28
5	Creamery butter	223·6	25·8	1·2	31·3	3·4	0·24
	December-January (winter)						
1	Hay-box (just boiled)	226·0	26·5	1·4	32·5	5·6	0·35
2	Simmering (just boiled)	226·4	26·2	1·3	34·4	4·4	0·29
3	Hay-box (boiled for 10 min.)	226·7	26·4	1·4	33·9	5·9	0·37
4	Simmering (boiled for 10 min.)	225·6	26·1	1·4	33·0	5·1	0·30
5	Creamery butter	223·5	25·0	1·3	31·4	3·3	0·26

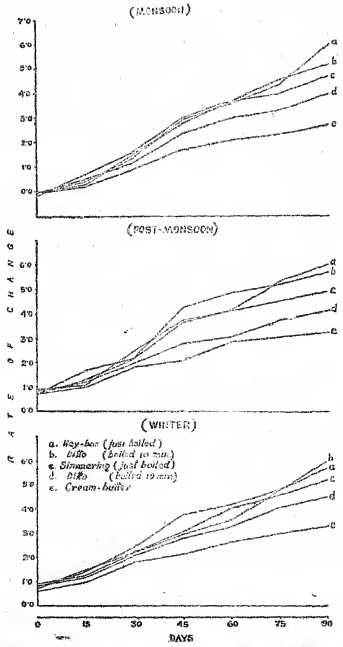

FIG. 1. The rate of change in peroxide value on storage

A perusal of the data in Table V suggests that prolonged storage does not materially affect the S. V., R. M., P. V. and I. V. of *ghee* samples prepared by different methods. The two constants which exhibit definite change are peroxide value and acidity. Both these values have significantly increased during the course of storage. The greatest increase in peroxide value is with hay-box (just boiled) sample followed by hay-box (10 min. boiled) simmering (10 min. boiled) and simmering (just boiled). The acid value is observed to follow the same order. The increase in these values is relatively greater in the samples prepared in the monsoon than in those prepared in the post-monsoon and winter month. These findings are supported by the trend of change in the peroxide value depicted in fig. 1, which also clearly indicates the comparative rate of deterioration in the storage quality of *ghee* samples prepared by different methods.

Summary

Investigation carried out to study the relative efficiency of 'hay-box' and 'simmering' methods in the preservation of morning milk shows that while the former method is more economical and obviates acquiring of smoky flavour in the final product, its efficacy as a means to preserve milk in all seasons is doubtful.

In both methods, the present practice of adding evening's unprocessed milk takes away much of the benefit derivable from processing and sweet preservation of the morning milk. Such addition does not ensure the type of fermentation desired in the course of ripening of milk with lactic starter and thus may eventually result in the production of poor quality butter and butter-milk.

The results of the present trials substantiate the claim made regarding higher outturn and initial quality of *ghee* by the hay-box method as against village simmering process. The keeping quality of 'hay-box' *ghee*, however, is inferior to that of *ghee* prepared by the simmering method.

Acknowledgements

The investigation was carried out under the guidance of the Director of Dairy Research, Bangalore with a financial grant from the Indian Council of Agricultural Research.

The Director, Veterinary Services, C. P. and his staff supervised the work and offered all facilities in carrying out the investigation at Jhallar Centre.

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INVESTIGATIONS ON FAMINE RATIONS
KANS (*SACCHARUM SPONTANEUM* LINN.)
A REORIENTATION IN ITS USE AS CATTLE FEED

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(Received for publication on 11 May 1948)

(With one text figure)

SINCE the publication of the first article on famine rations [Kehar, 1944], more information has become available on the quantitative and qualitative shortages of livestock feed. Ware [1943] pointed out that the total supply of roughage and concentrates in India (excluding States, for which accurate data were not available) was sufficient only for 78·83 and 29·1 per cent respectively of the adult bovine population. If this supply is calculated [Kehar, 1946] on the basis of physiological requirements of Indian cattle, weighing about 600 lb., the entire roughage and concentrates from all organized sources will suffice for only 106 and 35 million adult cattle respectively. This, however, does not take into account their milk production or growth requirements, nor the reqirements of 48 million sheep, 38 million goats, 4·5 million equines, 1 million camels, and 173 million fowls.

These shortages are not only of quantitative nature but also qualitative, because straws, which are nutritionally poorer than cultivated fodders, constitute more than 50 per cent of the total roughage supply. Even with normal rainfall the feeding-stuffs fall short of the livestock requirements. Since rainfall is rather uncertain in the tropics and in India three-fourth of the cultivated crop depends on it, acute scarcity of fodder results from drought caused by partial or total failure of monsoon or from floods caused by excessive rains. Both these conditions affect the productiveness of the soil and livestock are exposed to the ravages of famine. Hardly a decade passes without the visitation of a famine varying only in its intensity and it is known that since 1708 B. C. 105 famines have devastated India. During such droughts, tanks dry up and springs fail to reach the surface. In the 1937-40 famine—the severest within living memory—which affected Rajputana, Sind, and Western Punjab nearly 50 per cent of the animals died [Kehar, *loc cit*]. Jenkins [1944] reported that in the province of Bombay hardly a year passes without reports of insufficient rains and consequent crop deterioration or failure. He pointed out that the economic loss to the province as a whole was immense scarcity conditions.

The present investigations are an attempt to use hitherto unutilized sources of feeds to help animals tide over lean periods. The use of ripe *munj* and molasses [Kehar, 1944], mango-seed kernel [Kehar and Chanda, 1945] and entrails [Kehar and Chanda, 1946] has already been reported. Observations on the use of *kans* are presented in this article.

Kans is found practically all over India, specially in the plains, and particularly in the Central Provinces, Malwa plateau, and in the Terai zone of the United Provinces. It propagates both by seed and by under-ground spreading root-nodes. Under favourable conditions it reaches a height of about 10 feet, though usually it varies from 3-6 ft. It is very common on river banks, in damp depressions and swamps: but has also a remarkable adaptability to xerophytic areas and is often found colonising newly thrown-up sand banks. According to Bor [1941] it is found in the intensely moist portions of land adjoining rivers as well as in arid areas. Hole [1911] distinguishes three ecological forms of *kans*, (*a*) a dry sandy soil form, a xerophylous, type, (*b*) a swamp form, a hygrophylous type found in marshy swamps with an abundance of available moisture throughout the year and (*c*) a loam form intermediate between (*a*) and (*b*). *Kans* is commonly used for thatching huts and making brooms. Brown and Fischer [1918] in Philippines advocate its use for paper pulp. In

India, except for insignificant amounts used for thatching purposes and apart from the indifferent [Hall, 1946] and occasional browsing by animals, it is considered as one of the most pernicious weeds and large amounts of money are spent in certain farms on its eradication. Howard and Howard [1929] pointed out that in extreme cases of infestation the farmer had to give up cultivation of the particular land and Howard (1927) showed that *kans* was responsible for an enormous monetary loss in reducing the yield of cotton.

A survey of the *kans*-ridden areas suggested that it might be utilized as a famine fodder in green or dry condition. In both forms its chemical make-up (Tables I and VII) indicated that in green condition it compared favourably with some of the cultivated fodders and at the ripe stage with cereal straws. Encouraged by the above observations, it was considered desirable to study (1) its palatability in both dry and green conditions when fed as a single or mixed feed, (2) its nutritive value, (3) its effect upon the health of animals when fed over a prolonged period, and (4) yield of dry matter and other nutrients per acre.

Experimental

Since according to available information *kans* was not considered a normal fodder and was to be tried as a stand-by for scarcity periods, Kumauni bullocks, which were used as experimental animals, were kept for about 10 days on half the scheduled ration before commencing feeding of *kans*. The object was to simulate fodder shortage conditions prevailing in scarcity areas. The feeding trials were conducted at two different stages of growth of the plant, namely before blossoming in the green state and after it was dead ripe.

(1) Experiments on green *kans*

Green *kans* was analyzed for crude protein, ether extract, crude fibre, nitrogen-free extract, carbohydrates, calcium, and phosphorus. It was found (Table I) that it compared favourably with some of the cultivated fodders like guinea grass (prime) and napier grass (prime).

Table I

Chemical composition of kans *as compared with some cultivated fodders (on dry basis)*

—	Crude protein	Ether extract	Crude fibre	N.-free extract	Total carbo-hydrates	Calcium	Phos-phorus
Green *kans*	5·30	1·42	40·0	49·1	89·1	0·58	0·67
Guinea grass (prime) . . .	4·8	0·66	42·1	40·1	82·2	0·50	0·39
Napier grass (prime) . . .	5·4	1·89	31·9	44·2	76·1	0·33	0·35

It will be observed that its chemical make-up green *kans* is superior to guinea grass in protein, nitrogen-free extract, total carbohydrates and phosphorus, and to napier grass in nitrogen-free extract, total carbohydrates, calcium, and phosphorus.

For feeding trials three Kumauni bullocks weighing 382, 386 and 354 lb. were selected. Green *kans* at the pre-flowering stage was obtained every morning from the Institute farm. Prior to feeding green *kans*, these animals had free access to pasture and got other succulent cultivated fodder at night in the stalls. At first they did not relish the change-over to *kans*, but when the plant was chaffed the animals soon started taking a sufficient quantity. The mean dry matter consumption from this single feed (Table II) was 1·8 lb. per 100 lb. body weight.

TABLE II

Dry matter consumption of animals with green kans *as a single feed*

Animal No.	Body wieght lb.	Dry matter consumed from roughage gm.	Consumption of dry matter per 100 lb. of body weight lb.
39	382	3058	1·8
240	386	3583	2·0
408	354	2771	1·7

Nutritive value of green kans

After a preliminary feeding period of 30 days, a metabolism experiment was conducted according to the procedure detailed in a previous article [Kehar, *loc cit.*]. From the chemical composition it appeared that this grass might provide the maintenance requirement of animals and so no concentrate was fed. The results of the digestion trial are given in Table III.

TABLE III

The digestibility coefficient of green kans

Animal No.	Intake from green *kans* (gm.)	Excreted in fæces (gm.)	Amount digested (gm.)	Digestibility coeficient per cent	Average digestibility coefficient
			Crude protein		
39	162·1	65·9	96·2	59	
240	189·9	82·0	107·9	57	59
408	146·9	55·7	91·2	62	
			Ether extract		
39	43·4	20·7	22·7	52	
240	50·9	18·2	32·7	64	60
408	39·3	14·4	24·9	63	
			Crude fibre		
39	1223	294	929	76	
240	1433	400	1033	72	76
408	1108	230	878	79	
			Nitrogen free extract		
39	1501	622	879	59	
240	1759	745	10 4	58	61
408	1361	448	913	67	
			Total carbohydrates		
39	2724	916	1808	66	
240	3192	1145	2047	64	68
408	2469	678	1791	73	

The data in Table III show that the protein digestibility from *kans* is quite satisfactory. It is interesting to note that though animal No. 240 consumed a larger quantity of the roughage, the excretion in the fæces of undigested protein was more than that of the other two animals, the average digestibility coefficient of 57 per cent of protein is not much different from those of the individual data recorded. The digestibility of ether extract is about 20 per cent less in animal No. 39 while the other two showed concordant results. The digestibility of crude fibre is of great significance in ruminants and it may be observed that the average digestibility coefficient of crude fibre of green *kans* is as high as 76 per cent. All the animals behaved uniformly so far as the digestion of this constituent is concerned. An average digestibility coefficient of 76 per cent is generally found in good quality green feeds, like oats or maize. A variation of about 15 per cent is observed in the digestion coefficient of N-free extract and since there is little variation in the crude fibre digestibility between the individual animals, the total carbohydrate digestibility shows a variation of about 12 per cent only.

Nitrogen balance under green kans *feeding*

The nitrogen balance data shown in Table IV indicate that the animals had enough protein and that there was a considerable margin of safety when green *kans* formed the sole feed.

TABLE IV

Nitrogen balance under green kans *feeding*

Animal No.	Intake from green *kans* (gm.)	Excreted		Total excretion (gm.)	Balance (gm.)
		In fæces (gm.)	In urine (gm.)		
39	25·93	10·60	6·84	17·44	+8·49
240	30·38	13·12	5·86	18·98	+11·40
408	23·50	8·91	5·54	14·45	+9·05

When starch equivalent and total digestible nutrients are calculated from the above digestibility figures, *kans* is found to contain 19·3 lb. of starch equivalent and 65·6 lb. of total digestible nutrients.

In comparing the digestibility coefficient of *kans* (Table V) with some of the green feeds it was observed that the amount of digestible protein of 3·13 lb. per 100 lb. of *kans* is slightly lower than that obtained from an equal amount of *bajra*, elephant grass, guinea grass or maize, but the amount of digestible carbohydrate is more than that of *bajra*, elephant grass and guinea grass, and a starch equivalent value of 19·3 is considerably higher than those of all the above-mentioned green feeds.

TABLE V

The digestibility coefficient and nutritive value of green kans *as compared with some cultivated fodders*

Feed	Origin	Digestibility coefficient				Digestible nutrients per 100 lb. of raw material				
		C.P.	E.E.	C.F.	N.F.E.	Total carbo-hydrates	C.P.	Total carbo-hydrates	E.E.	S.E.
Green *bajra*	Lyallpur	62	67	60	69	..	4·31	52·63	1·02	11·3
Elephant grass	Lyallpur	62	59	63	65	..	3·35	48·54	1·33	9·6
Guinea grass	Bangalore	58	43	61	52	59	4·44	45·23	0·73	7·9
Green maize	Lyallpur	61	65	70	76	..	4·68	60·94	0·96	13·1
Green *kans*	Izatnagar	59	60	76	61	68	3·13	60·59	0·85	19·3

Calcium and phosphorus balances.

It has already been stated that green *kans* is a maintenance ration in regard to both energy and protein. A study of the Ca and P balances of animals under the same condition of feeding was also attempted. A perusal of the data in Table VI shows that all the animals were in positive balance with regard to these minerals.

TABLE VI

Calcium and phosphorus balances

Animal No.	Intake (gm.)	Excretion		Total (gm.)	Balance (gm.)
		Fæces (gm.)	Urine (gm.)		
		Calcium			
39 . . .	17·7	10·6	1·6	12·2	+5·5
240 . . .	20·8	12·3	1·1	13·4	+7·4
408 . . .	16·1	8·5	1·5	10·0	+6·1
		Phosphorus			
39 . . .	20·5	4·8	0·5	5·3	+15·2
240 . . .	24·0	4·7	0·7	5·4	+18·6
408 . . .	18·6	3·4	0·6	4·0	+14·6

Experiments on ripe kans

Nutritive value of the ripe kans.—The chemical composition of ripe *kans* used for feeding trials is given in Table VII.

TABLE VII

Chemical composition of ripe kans *as compared with some common cereal straws*

——	C.P.	E.E.	C.F.	N.F.E.	Total carbohydrates	Ca.	P.
Ripe *kans* . .	3·35	1·16	40·2	48·0	88·2	0·42	0·15
Wheat straw . .	3·30	1·16	38·5	45·2	83·7	0·30	0·07
Rice straw .	2·90	0·86	33·4	45·6	79·0	0·36	0·06
Ragi straw . .	3·70	0·92	35·9	51·4	87·5	0·80	0·07

It will be observed that from its chemical composition *kans* is as good as wheat straw. It seems to be superior to rice straw in almost every respect but contains less protein and calcium than *ragi* straw. Unlike *kans* at the green stage, ripe *kans* contains much less crude protein and thus by itself cannot form a maintenance ration. A small supplementation with protein-rich concentrates is, therefore, needed to make the whole ration adequate in both energy and protein. Three adult Kumauni bullocks were fed about $\frac{1}{2}$ to $\frac{3}{4}$ lb. of rape cake in addition to chaffed *kans ad lib.* The dry matter consumption as seen from Table VIII is 1·5 lb. per 100 lb. body weight.

TABLE VIII

Dry matter consumption of three bullocks fed with rape-cake in addition to kans

Animal No.	Body weight	Dry matter consumed from roughage	Dry matter consumed from cake	Consumption of dry matter per 100 lb. of body weight
	lb.	gm.	gm.	lb.
39	388	2037	354	1·4
177	288	1858	266	1·6
193	280	1800	266	1·6

A metabolism experiment was conducted after a preliminary experimental period of about a month. The results are given in Table IX.

TABLE IX

Digestibility coefficient of ripe kans *fed with rape-cake*

Animal No.	Intake from *kans* gm.	Intake from cake gm.	Total intake gm.	Excreted in fæces gm.	Amount digested gm.	Digestibility coefficient per cent	Mean
			Crude protein				
39	68·2	125·1	193·3	78·4	114·9	59	
177	62·1	94·0	156·1	73·8	82·3	53	53
193	60·3	94·0	154·3	79·8	74·5	48	
			Ether extract				
39	23·6	38·8	62·4	22·1	40·3	65	
177	21·6	29·2	50·8	19·0	31·8	63	63
193	20·9	29·2	50·1	19·3	30·8	62	
			Crude fibre				
39	818·9	29·4	848·3	274·5	573·8	68	
177	746·9	22·1	769·0	261·4	507·6	66	66
193	723·6	22·1	745·7	262·3	483·4	65	
			Nitrogen-free extract				
39	977·8	118·6	1096·4	560·8	535·6	49	
177	891·8	89·1	980·9	674·3	306·6	31	36
193	864·0	89·1	953·1	679·1	274·0	29	
			Total carbohydrate				
39	1797	148	1945	835	1110	57	
177	1639	111	1750	936	814	47	50
193	1588	111	1699	941	758	45	

The results show that in respect of crude protein, N-free extract, and total carbohydrate, two of the animals showed very satisfactory results while animal No. 39 recorded an increased digestibility coefficient in all these fractions. The crude fibre digestibility was, however, uniform, and this was so with ether extract also. If the digestibility coefficients of *kans* at both the green and ripe stages are compared, it will be seen from Tables VIII and IX that when ripe *kans* is fed together with a suitable amount of cake, the values for ripe and green *kans* are almost identical with regard to digestible fractions of crude protein, ether extract and crude fibre, whereas the digestibilities of N-free extract and total carbohydrate are invariably higher when green *kans* is fed as a single feed.

It may also be seen from the figures given below that the starch equivalent values of both green and ripe *kans* are practically the same, whereas the amount of digestible protein is considerably greater in the green stage. The excess of digestible protein available in 100 lb. of green *kans* as compared with the ripe stage is equivalent to 3·3 lb. of rape cake.

100 lb. of ripe *kans* (Dry Matter, 85 per cent)		100 lb. of green *kans* (Dry Matter, 46 per cent)	
D.P. lb.	S.E. lb.	D.P. lb.	S.E. lb.
0·3	20·0	1·44	19·3

The digestibility coefficient of ripe *kans* as compared with other cereal straws is shown in Table X.

TABLE X

Digestibility coefficient of ripe kans and *cereal straws*

——	C.P.	E.E.	C. fibre	N.F.E.	T.C.	S.E.
Ripe *kans*	11	30	66	35	49	20
Rice straw	0	47	61	42	51	18
Wheat straw	0	36	61	53	56	22

It will be observed that the digestibility coefficient of crude protein of ripe *kans* is far superior to that of wheat or rice straws, whereas the digestibilities of crude fibre and total carbohydrates are almost as great as those of the other straws. In energy value also it is as good as wheat or rice straw.

Nitrogen balance

The nitrogen balance under ripe *kans* feeding was also calculated. It will be observed from Table XI that when ripe *kans* is supplemented with ½ to ¾ lb. of cake, the ration is adequate for maintaining the nitrogen balance.

TABLE XI

Nitrogen balance under dry kans *and rape-cake feeding*

Animal No.	Intake from dry *kans*	Intake from rape-cake	Total intake	Excreted in faeces	Excreted in urine	Total excretion	Balance
39	10·92	20·00	30·92	12·64	13·28	25·92	+5·00
177	9·96	15·03	24·99	11·72	12·80	24·52	+0·47
193	9·65	15·03	24·68	12·67	12·16	24·83	−0·15

Calcium and phosphorus balances

All the three animals showed a negative calcium balance, while the extent of negative phosphorus balance was negligible. It is thus desirable that in feeding ripe *kans* calcium should be supplemented. The balance data are shown below.

TABLE XII

Ca and P balance under ripe kans feeding

Animal No.	Intake			Output			Balance
	Kans	Cake	Total	Feces	Urine	Total	
				Calcium			
39	8·56	3·01	11·57	11·71	2·30	14·01	−2·46
177	7·80	2·26	10·06	10·65	1·90	12·55	−2·49
193	7·56	2·26	9·82	10·48	1·74	12·22	−2·40
				Phosphorus			
39	3·06	4·35	7·41	7·22	0·40	7·62	−0·21
177	2·79	3·42	6·21	5·78	0·50	6·28	−0·07
193	2·70	3·42	6·12	5·90	0·60	6·50	−0·32

Considering the enormous potentialities of *kans* and its possible use in future as a fodder on a large scale, a long term experiment was conducted from 1 January 1940 to 10 August 1940, on dry *kans* as the sole source of roughage balance by supplementation with wheat-bran, cake and molasses. Two Kumaoni bulls were used for the experiment. Since this fodder was originally intended for scarcity periods, when animals could not be supplied with the normal amount of food, these experimental animals were kept on 75 per cent of their ration for 10 days. The mean weight of the group (Fig. 1) then fell from 280 lb. to 246 lb. They were then fed the experimental ration consisting of 6 lb. of chaffed ripe *kans*, 1 lb. molasses, ½ lb. wheat-bran, and ½ lb. mustard-cake. The chaffed *kans* was moistened with water 24 hours before offering to the animals. Then molasses, mixed with equal weight of water, was sprinkled over the roughage. The animals began to relish it after a couple of days and regained their normal weight within 15 days. During the following 10 days they gained 8 lb. more than their original weight. At this stage Izatnagar was passing through a cold wave, and as the animals could not be furnished with bedding on the cement-floored stalls for fear of their eating it, they lost a few pounds, but they resumed the weight increase immediately the cold wave was over till they reached a peak showing an increase of 30 lb. on the 150th day of the experimental feeding period.

In order to determine whether the animals on return of favourable monsoon would be useful for work on this ration, they were sent to the farm for about four hours' work every day from the 105th day to the 190th day of the experiment. They then maintained their body weights and were in fairly satisfactory state of health.

Meanwhile two more kumauni bullocks were placed on the same ration on the 55th day of experimentation. They started relishing this ration from the beginning and by the 120th day their weight had increased from 244 lb. to 272 lb. Unfortunately at this stage one animal developed fever and had to be discontinued and was replaced after 10 days by another. The mean weights of the two animals on the 130th day of the experiment was 251 lb., which rose to 278 lb. on 210th day when feeding was discontinued. From the above long term feeding trial extending over a period of about nine months it is evident that ripe *kans* can be fed as a roughage without adverse effect.

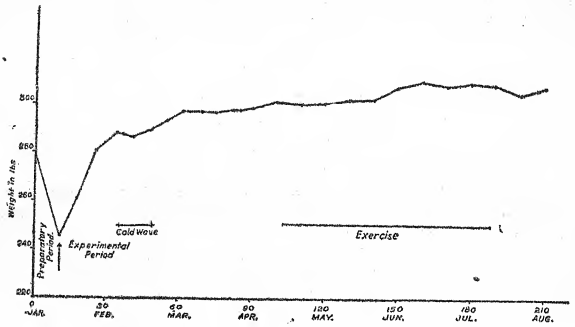

FIG. 1. Average weight of two Kumaoni bullock under '*Kans*' feeding.

Amount of kans *available in India.*—It has not been possible to obtain precise information on this point, nor of the percentage that is satisfactorily utilized. The Chief Conservators of Forests and Directors of Agriculture of different provinces were asked to supply information as to the exact acreage under *kans*, along with its approximate yield but the replies were disappointing. Whereas all the officers reported that *kans* grows over large areas the amount of *kans* per acre was reported to vary from two md. in Assam to 100 md. in the Punjab. Nowhere has systematic work been done on the exact yield of *kans* per acre under a given set of conditions. In order to arrive at an approximate estimate, a plot measuring 65 feet square was selected at the Clutterbuckganj nursery through the courtesy of Mr. Kanjilal, Silviculturist, U. P., and the Chief Conservator of Forests, the United Provinces since the intention was to record the maximum yield the plant was cut when it reached the pre-flowering stage or a height of about 3 ft. Four cuts could be obtained in a year. The date of cutting and the yield per cut along with the chemical composition are given in Table XIII.

TABLE XIII

Samples of kans *collected at different intervals*

Date	18-8-45	28-9-45	13-6-46	15-8-46
Amount of green *kans* in lb.	292	209	284	240
Total ash	10·56	9·76	23·65	15·51
Ether extract	1·54	0·62	1·04	3·19
Crude protein	4·12	6·95	3·47	4·60
Crude fibre	35·44	38·72	27·27	35·13
Nitrogen-free extract	48·34	43·95	44·57	41·57
CaO	0·61	0·52	1·20	0·60
P_2O_5	0·56	0·72	0·72	0·82

It will be observed that the total annual yield per acre under Clutterbuckganj conditions with an average rainfall of about 30 in., is 10568 lb. (4861 lb. dry matter) containing 2039 lb. starch equivalent and 3189 lb. of total digestible nutrients.

Samples from all these four cuts were analyzed and it was found that if 4 per cent. crude protein, 0·4 per cent CaO and 0·5 per cent P_2O_5 are taken as the criteria of a good quality fodder, green *kans* warrants a place in that category.

The foregoing observations indicating green *kans* at the pre-flowering stage to be equivalent to some of the cultivated fodders and ripe *kans* equal to some of the common cereal straws, will introduce a reorientation in our views about the use of *kans*. Although these experiments were originally intended to make available some feeds to cattle under famine conditions, on the basis of the results reported above it can be safely recommended as a normal cattle feed.

SUMMARY

In an effort to exploit hitherto unutilized sources of feeding stuffs, attempts have been made to find it *Saccharum spontaneum* (*kans*), which is not economically used much at present and is considered a pest, can be used as cattle feed during famine periods. On chemical analysis *kans* was found to be fairly rich in protein and other constituents. Feeding trials on the pre-flowering and dead ripe stages of *kans* indicated that :

1. Green *kans* at the pre-flowering stage forms a maintenance ration and can be fed as a single feed. In regard to chemical composition and nutritive value it compares favourably with some of the cultivated fodders like guinea grass and napier grass.

2. Ripe *kans* compares favourably with common cereal straws like wheat and rice straw. The digestibility coefficient of protein of *kans* is far superior to that of wheat or rice straws.

3. Ripe *kans* roughage fed for about eight months showed no adverse effect on the health of animals.

It has not been possible to ascertain the total quantity of *kans* available in India, but from information obtained from the various provincial Chief Conservators of forest and Directors of Agriculture, it is assumed that it grows in every province over millions of acres. Each acre roughly produces about 10568 lb. of green *kans*, giving 2039 lb. of starch equivalent and 3189 lb. of total digestible nutrients.

Although these observations were originally intended to find some feed for starving animals to enable them to tide over drought periods, these findings indicate that *kans* can be recommended as a normal cattle feed.

ACKNOWLEDGEMENTS

The author thanks Dr S. K. Talapatra and Mr. R. Chanda for technical assistance.

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INVESTIGATIONS ON FAMINE RATIONS *CARTHAMUS OXYCANTHA* Bieb (*KANTIARA*)

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(Received for publication on 25 February 1947)

According to a recent estimate the quantity of roughages available in India falls short of the requirement of the livestock population by about 45 per cent. This shortage is felt all the more when there is a partial or total failure of monsoon. Wars and famines have multiplied needs and created further shortages. To meet this quantitative insufficiency, efforts have been made to utilize so far unexploited fodder resources for animals. Observations on '*munj*' *Saccharum munj* and molasses [Kehar 1944] and on *Kans* (*Saccharum spontaneum*)—[Kehar 1946] have already been reported. This article, presents observations on the use of *Carthamus oxycantha* Bieb as a fodder for livestock.

Carthamus oxycantha— (*Kantiara, Miankalai*—Punjab; *Kuzbural, Khareza*—Trans-Indus; *Kantila*—U. P.) is a native of the dry and arid tracts of North India. It grows wild in the United Provinces, Punjab, Baluchistan, Afghanistan and westwards to the Caucasus during the months of May and June. The spinosed clumps constitute an objectionable feature of the grassy tracts. The village herds even though they have nothing else to graze during the hot months of May and June, would not eat the luxuriously growing '*Kantiara*' due to its spinosed leaves. In the early stages, however, *Kantiara* has no spines and in some parts of the country the weeds from wheat fields which include *Kantiara* also, are fed to cattle with chaffed *Jowar* wheat straw.

Experimental

The plant was chemically analyzed and as shown in Table I, it contains a high percentage of protein and calcium. Toxicological analysis gave no indication of the presence of any component of toxic nature.

Table I

Chemical composition of 'kantiara'

(*Percentage on dry basis*)

	Crude protein	11·03
	Ether extract	1·23
	Fibre	22·31
	Nitrogen-free-extrant	51·53
	Total ash	13·90
Carotene	Calcium	1·30
	Phosphorus	0·133
	Plant (excluding roots)	16·7%
	Leaf and flower	9·45%
	Stalk and little of leaf	2·09%

Inspite of its richness in the nutritive components, it was extremely difficult to feed the plant to the animals on account of its sharp spines. After several trials and failures, an easy and workable method acceptable to the animals was found out. The spines were rounded off by beating the dry plants with thick wooden sticks (commonly known as *mugris*), until the material, when pressed in the hand, would no longer prick.

The plants, after rounding off their spines, were placed before the animals. They showed no disinclination but did not consume their full quota of dry matter. It was, therefore, supplemented with molasses and it was observed that the dry matter consumption rose to about 1·75 lb. per 100 lb. body weight. Two adult Kumauni bullocks were then fed for a period of 15 weeks on a ration consisting of 2¼ lb. barley straw, 2¼ lb. *Kantiara* ½ lb. cake and ½ oz. salt. The quantity of barley* straw and *Kantiara* was soon raised to 3 lb. each. The animals presented a healthy appearance and the average body weight of the animals rose from 233 lb. to 245 lb. during the observation period.

Two more adult animals were then fed on a ration* consisting of *Kantiara* and oat hay in equal proportion. After the first three weeks of the observation period both the animals suffered from a severe infection of 'foot and mouth' and lost 22 lb. in weight. On recovery, they started picking up and gained an average weight of 10 lb. during the observation period of ten weeks.

Kantiara when young, say up to six inches in height, has no spines and can therefore be fed to animals, as such. Young *Kantiara* (below six inches) mixed with wheat *bhusa* was next fed to a set of three adult bullocks for a period of five weeks. Towards the end of the period a metabolism trial was conducted by the procedure detailed in a previous article [Kehar 1944]. Two of the animals fell victim to 'foot and mouth' disease and the trial could, therefore, be conducted on one animal only. The nitrogen, calcium and phosphorus balance was found to be positive and the animal gained 44 pounds in weight.

Another feeding trial was next conducted on three adult bullocks. A mixture of dry *Kantiara* and wheat *bhusa* in the proportion of 1 : 2 was fed to them. The proportion of *Kantiara* was gradually raised and wheat *bhusa* withdrawn until the ration contained 60 per cent of *Kantiara*. The ration finally given to each animal consisted of ½ lb. rape cake, 4 lb. *Kantiara* and 2 lb. wheat *bhusa*. In addition to the above, each animal was given one oz. of common salt daily.

The ration was fed for a period of eight weeks. Average dry matter consumption was 1·8 lb. per 100 lb. body weight. It will be observed from Table II that two of the animals gained in weight while the third showed a slight decrease. The net result was an average increase of 7·3 lb. in body weight on the experimental ration.

Table II

Live weight record of animals

(*Weight in pounds*)

Date	Animal No. 239	Animal No. 240	Animal No. 267	
3rd January 1946	256	364	276	
(Date of first feeding)				
7th January 1946	260	364	280	
14th January 1946	260	360	284	
21st January 1946	268	362	280	
23rd January 1946	268	362	280	Metabolism period
2nd February 1946	264	364	278	Metabolism period
4th February 1946	264	364	278	
12th February 1946	256	384	268	
18th February 1946	264	378	268	
25th February 1946	260	384	264	
27th February 1946	264	384	270	
Change in weight	+8 lb.	+20 lb.	−6 lb.	
Average gain in weight 7·3 lb.				

* In both these feeding trials two pounds of green fodder was fed twice a week to provide carotene

The animals all along presented a healthy appearance. After about three weeks of feeding on this ration a metabolism experiment was conducted on the animals. The results showing the digestibility coefficient, nitrogen, calcium and phosphorus balance of the whole ration and the digestibility coefficients of *Kantiara* are given in Tables III, IV, V and VI, respectively.

TABLE III

Digestibility coefficient of whole ration

Animal No.	Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Total carbohydrate
239	46·0	52·0	56·1	53·7	52·5	50·9	51·4
240	42·6	46·5	57·3	54·9	55·4	39·4	44·8
267	43·9	50·5	59·2	61·1	48·0	49·5	62·8
Mean	43·9	49·7	57·5	56·6	52·0	46·6	53·0

TABLE IV

Nitrogen balance

Animal No.	*Kantiara*	Intake (gm.)		Total	Excretion (gm.)		Total	Balance (gm.)	Biological value
		Rape cake	Wheat bhusa		Faeces	Urine			
239	27·71	10·31	4·70	42·72	18·75	12·21	30·96	+11·76	72·63
240	25·07	10·31	4·65	40·03	17·10	17·92	35·02	+5·01	57·85
267	24·65	10·31	4·65	39·61	16·15	15·04	31·19	+8·42	64·33

TABLE V

Calcium and phosphorus balance

Animal No.	Calcium (gm.)			Phosphorus (gm.)		
	Total intake	Total excretion	Balance	Total intake	Total excretion	Balance
239	24·94	18·75	+6·19	5·95	4·77	+1·18
240	22·98	18·16	+4·82	5·74	5·24	+0·50
267	22·63	17·53	+5·10	5·70	4·38	+1·32

TABLE VI

Digestibility coefficient of Kantiara

Animal No.	Crude protein	Ether extract	Nitrogen free extract
239	54·9	27·9	45·9
240	56·5	27·8	51·0
267	59·6	40·3	36·1
Mean	57·0	32·0	44·3

It will be observed from the Tables given above that the replacement of wheat *bhusa* by *Kantiara* to the extent of 66 per cent gives a wide positive nitrogen and calcium balance. The phosphorus balance too is fairly positive. The digestible nutrients per 100 lb. of dry *Kantiara* as compared with other straws is given in Table VII.

TABLE VII

Digestible nutrients of Kantiara *as compared with other straws*

	Digestable protein	Starch equivalent	Table digestible nutrients
Kantiara	6·3	20·78	34·1
Wheat *bhusa*	0·0	21·9	48·0
Rice straw	0·0	27·1	49·5
Jowar straw (Chittral)	1·5	30·2	48·2
Jowar straw (Periamanjal)	0·11	29·5	53·4
Ragi straw	..	38·4	56·9

From the above figures it appears that the digestible protein in *Kantiara* is much greater than in any of the straws. As regards its starch equivalent, it compares favourably with wheat *bhusa* but is inferior to rice, *jowar* and *ragi* straws. The amount of extra foodstuff, in terms of starch equivalent, digestible protein, and total digestible nutrients available from this source per acre of land is given in the Table VIII below.

TABLE VIII

Yield of kantiara

Dry matter	Starch equivalent	Digestible protein	Total digestible nutrients
	Lb.	Lb.	Lb.
1200 lb.	249·36	75·6	409·2

It has been observed that one man working for eight hours a day, takes three days to collect the entire *Kantiara* yield from one acre; thus the total cost of the foodstuff available from *Kantiara* per acre of land works out to about Rs. 3.

SUMMARY

Investigations have been made to find if *Kantiara* could be utilized as a fodder for livestock in times of scarcity. It was found that the plant is fairly rich in nutritive components like crude protein and calcium and possesses no toxic components. Still, the plant, except when very young (below six inches in height) cannot be fed as such to the animals on account of its spinosed leaves. An easy method for making it fit for the consumption of animals has been suggested.

A mixture of *Kantiara* with various supplements like wheat *bhusa*, oat hay and molasses was fed to different groups of animals for periods ranging from 5-15 weeks. The animals presented a healthy appearance with apparently no untoward effect.

The crude protein percentage in the plant is superior to any of the straws and when fed with wheat *bhusa* gave a positive nitrogen, calcium and phosphorus balance. With green *Kantiara* and wheat *bhusa* the animal was found to have gained 44 lb. in weight, while with dry *Kantiara* and wheat *bhusa* the average gain in weight was 7·3 lb.

It is suggested that *Kantiara* may be used with advantage as a supplement fodder in times of scarcity. The fairly high value of the digestibility coefficient of protein and nitrogen feed extract give it a prominent place in the list of straws.

The amount of extra foodstuff available from this source has been estimated and from the figures given in the text it will be seen that it is found in considerable quantity at a negligible cost.

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STEPHANOFILARIASIS AMONG BUFFALOES IN ASSAM*

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(Received for publication on 21 April 1948)

THIS article is based on an investigation into an outbreak of Stephanofilariasis among buffaloes in Nowgong district, Assam, and it also records the observations on its occurrence in other parts of the province. Fairly large number of buffaloes are maintained in certain localities in Assam and the climatic and geograhpical conditions seem to suit them well. Stephanofilariasis or ear-sore as it is popularly called, was investigated in buffalo herds of several localities in five districts. The incidence of the disease has also been reported from other parts of India, particularly from Bihar, Orissa and Madras. The clinical lesion observed in the ear of buffaloes is known by various names, such as, ear-sore, Enzootic Otorrhoea, Otitis, Otacariasis, Contagious otorrhoea, etc. It seems probable that the condition is likely to be caused by other factors or agents than *Stephanofilaria Sp.*, depending upon the locality, climate and enviornment. However, in this paper, the specific nature of the parasite occurring in the ear-sore of buffaloes in Assam, is recorded and so it is considered appropriate to designate the condition as Stephanofilariasis.

Microfilariae or the larval forms of certain adult nematode worms, are found free in circulating blood as well as in skin lesions of domestic animals in India. It is not uncommon to demonstrate the presence of microfilaria in the peripheral circulation, causing no apparent inconvenience to the animal. However, the occurrence of microfilaria as a morbid entity of importance affecting the health of some species of domestic animals in this country, has been recorded by some workers during the past few years.

In a resume on the subject Sen [1931] stated that only two species of microfilaria were definitely recorded in equines and pointed out that the occurrence of microfilariasis in the horse as a condition concomitant with other forms of blood infection had been the general observation of workers. In dogs microfilariasis is commonly noticed with variable symptoms—from being devoid of noteworthy symptoms to being fatal. Datta [1935] indicated that his studies on the histopathology of hump-sore in cattle, had led him to conclude that this disease of Indian cattle was similar to 'Cascado' of cattle in Dutch East Indies, caused by *Stephanofilaria dedoesi*. Pande [1935, 1936] detected the occurrence of and recovered microfilaria and live adult worms from the lesions of hump-sore among cattle in Assam. After pointing out certain anatomical differences between *S. dedoesi* and the new parasite, he designated it as *Stephanofilaria assamensis*, a new species, and attributed its constant presence in or association with the morbid skin lesions, to be the cause of hump-sore in cattle. While elucidating the cause of subcutaneous haemorrhagic nodules in cattle, a disease of economic importance in this country, Bhalerao [1939] pointed out that the worm affecting cattle was *Parafilaria bovicola*. Srivastava [1939] reported the occurrence of cutaneous microfilariasis in a bullock with a description of the clinical features of the disease as it occurred among cattle in Bombay province. Nathani and Sankaranarayanan [1940] have demonstrated the presence of microfilaria in a chronic indolent sore in the sheath of a breeding bull that ultimately responded to treatment with antimosan. Recently, in an interesting survey on applied helminthology in India, Bhalerao [1947] has dealt with the occurrence and also new findings of some important parasites including filariid worms of man and animals. He also refers to the attempts made to discover the insect vector of *S. assamensis* causing hump-sore of cattle. It is evident that much attention is being paid, in recent years, to disease conditions among livestock caused by helminths. In this paper the occurrence of Stephanofilariasis as a specific disease entity in buffaloes causing ear-sore, is described.

* Paper read at the Indian Science Congress (Section of Medical and Veterinary Sciences), held at Patna in January 1948.

NATURE OF OCCURRENCE

The disease occurs generally in a sporadic form. A few buffaloes in a herd are affected and there is hardly any indication either for a slow or for a rapid spread to other animals. Stray cases in a village are also observed. However, it occasionally assumes the form of an outbreak affecting a large number of buffaloes in a village and even spreading to a few villages in an area having large buffalo population. In such cases, the prevalence of the disease is seasonal; the outbreaks being noticed in the rainy season, especially soon after the first showers in February and March. But stray cases, not of acute or severe type, have been observed in almost all the months of the year. It seems that the lesions are quiescent in winter season and become active during rains and in this respect the behaviour is similar to that of hump-sore of cattle.

The occurrence of the disease is observed in all the districts in the plains of Assam, particularly in lowlying localities on either bank of the Brahmaputra and the Surma rivers and their major tributaries. In fact, the first investigation of affected cases was carried out in silchar on the bank of the Barak river, a tributary to the Surma, in Cachar district in November, 1939. Subsequently the disease was investigated in several localities of other districts, all of them situated on the bank of rivers. Cases are often seen in herds that are usually maintained in marshy low-lying areas or in riverain tracts that abound in grazing pastures. The occurrence of the disease is more common in the Surma Valley and the Lower Assam Valley than in the Upper Assam Valley. It is noteworthy that the disease has not been observed in buffaloes in the hill districts of the province.

Buffaloes of both sexes and of all ages seen equally susceptible. But more cases are often seen among the adult and old animals. The disease is very rarely fatal even though the incidence of affected animals in an outbreak may vary from 20 to 50 per cent depending upon the severity of the outbreak.

Outbreak in Nowgong district

Investigation into this condition was carried out in March 1942 and in subsequent years in Kampur, Nowgong district. In the first instance, it prevailed in the form of an epidemic affecting about 30 buffaloes in Kampur and two adjoining villages. The outbreak was noticed about ten days after the rains. The first case was observed at Kampur in the middle of March and subsequently more cases were reported in the three villages.

The villages are situated on either bank of a river that winds its way near Kampur. The whole area is low-lying and is usually flooded during rains. Fairly extensive grazing area is available in the marshy banks of the river and around low lying pools where buffaloes graze and lie down for rest in the stagnant water for some part of the day.

Symptoms

Frequent shaking of the ear and sometimes slightly offensive odour near the animal, are first observed. Examination reveals the inflamatory condition at the base of the ear near about the meatus while the external ear becomes more or less thickened presenting cracked surface of the skin. Appetite is diminished and there is definite decrease in milk yield. The inflammed area shows numerous minute pin-point congested spots. Frequent rubbing or scratching of the ear to poles or trees is often seen. One or both the ears are affected. If one ear is affected the animal holds its head side ways with the diseased ear down.

Slight watery discharge is observed at the early stage which later becomes purulent, light or dirty yellow in colour with offensive odour. Sometimes ulceration of lining of the ear is seen. The exudate is occasionally streaked with blood. There is usually extraneous infection. The animal shows much pain when handled. Occasionally pharyngitis and congestion of the nasal mucous membrane with profuse discharge, are noticed. Submaxillary lymph glands become sowllen. Neglected cases become fly-blown and even maggots are formed. In severe cases, when both ears are affected there is constitutional disturbance which interferes with the working capacity of the

animal. A large number of cases are usually seen in working animals *i.e.*, male buffaloes. They are mainly used for field work such as ploughing and for carts. During the rest period of the day they often remain in miry and marshy places near water-ways. This habit, to a certain extent, seems to retard the improvement in treated cases. The condition usually becomes chronic and often remains quiescent in the cold season. Generally active lesions reappear during rains, the monsoon weather being most favourable.

Several cases were examined in various localities during the rainy and winter seasons, to study the differential characters of the lessions in the active and quiescent stages. The symptoms described above refer to the active stage in the rainy season. In winter, the lesions become inactive and appear more or less dry. The surface presents a healthier appearance and there is less local irritation. In fact, the animal does not manifest any inconvenience or pain. Parasites have been recovered from all the cases though their number from these cases may be less. When the lesions are active, more parasites are obtained from the scrapings.

The disease usually runs a slow course of two to three weeks or more. Generally one ear is affected and sometimes both are involved. Recovery ordinarily takes place and there is rarely any mortality.

Collection of scrapings

Scrapings are collected from the affected part after cleaning the surface with normal saline solution. A blunt scalpel or a curette will serve the purpose. Certain observations regarding the collection of adult worms from fresh scrapings are worthy of note. While scraping affected areas or lesions, it is not uncommon that a portion of the parasite protrudes from the scraped surface. Dropping a small quantity of saline over the area, removes the oozing blood, thus exposing more distinctly the adherent parasite which can be gently removed with a pair of forceps or camel-hair brush. Besides, when scrapings are collected in normal saline in a Petri-dish, the worm could be found embedded in a piece of scraped tissue, sometimes with a portion of it protuding. A careful teasing of the clumps of the scrapings dislodges the worm. While examining the worm under microscope, it is not infrequent that the anterior extrimity is covered with fragments of tissue.

Laboratory findings

Scrapings and smear preparations were examined in all the cases that were investigated. From the scrapings adult nematode worms, both male and female, have invariably been recovered for examination. As usual in Filariidae, female parasites were found longer than the male ones. Smear preparations have revealed numerous microfilariae. These preparations have been examined fresh as well as stained with Leishman stain. In wet smears, the microfilaria is coiled up inside an egg-like membrane. In stained smears, free forms are detected. The morphological characters of the parasite have been found apparently identical with those of *Stephanofilaria assamensis* causing hump-sore of cattle as recorded by Pande [1936].

Further, with the object of getting the findings confirmed, collection of worms from the ear scrapings was sent to the Officer in-charge, Veterinary Zoology Section, Imperial Veterinary Research Institute, Mukteswar, and it has been identified as belonging to *Stephanofilaria (sp)*. Besides, specimens of worms from further cases were sent to the Officer-in-charge, Helminthiasis Scheme, Imperial Council of Agricultural Research, Lucknow University, Lucknow, and these too were identified as *Stephanofilaria (sp)*.

In the smear preparations, in addition to microfilaria, various organisms have been seen, such as cocci, fungi, acari parasites, etc. These are considered saprophytic or secondary invaders in view of the anatomical position of the parts affected ; nor is their presence constant in all cases.

Transmission of the disease

Experimental transmission or reproduction has not been successful so far. In view of the seasonal occurrence of the disease, insects must be considered to be the probable vectors of the

causative parasite. During the rainy season when the lesions become active, the sores are swarmed with flies—*Stomoxys calcitrans* and *Musca* (*sp*). But nothing definite regarding transmission could be ascertained. It may, however, be mentioned that the probable vector or intermediate host of *Stephanofilaria* (*sp*). causing skin affections of cattle in this country and elsewhere, is yet undetermined.

Treatment and control

During the period of investigation of this disease, experimental treatment of hump-sore in cattle was started in October, 1940, using tartar emetic, due to its recognized parasiticidal properties. Local application of tartar emetic ointment, 1 in 25, gave indications of improvement in the skin lesions and its wider application in all types of cases of hump-sore gave promising results. Since the casual agents of hump-sore and ear-sore appeared identical, excepting for the difference of host and seat of predilection, the same method of treatment was adopted for the lesions in the ear. The results were very encouraging and in fact, it was found that the response was more satisfactory in ear-sore than in hump-sore.

Application of tartar emetic ointment is adopted as a routine treatment now-a-days in Assam, for ear-sore in buffaloes. After dry cleaning and removal of superficial debris, tartar emetic ointment 1 in 25 (one dram of tartar emetic powder thoroughly mixed with three ounces of vaseline), is rubbed well over the affected part. Daily dressing is continued for three days followed by a rest period for three days. The treatment is repeated for three days more with an alternative equal period of rest. In two to three courses of treatment satisfactory clinical recovery is observed. The alternate rest period was found necessary due to the irritant property of the drug. No other medicine was tried as tartar emetic appeared safe, effective, cheap and simple to use.

With proper application of this treatment, improvement in the condition of the sore is noticed in the course of one to two weeks. It is necessary to avoid damage by external agencies, like pecking by crows and excessive fly infestation. The habit of remaining in water or marshy pond should be avoided to keep the sore dry and clean.

Discussion

It is well-known that nematodes of *Stephanofilaria* (*sp*). have been associated with skin affections of cattle, most probably as etiological factors, in 'Cascado' of cattle in Dutch East Indies [1933], in sore-like skin lesions of North American cattle (1934), and in hump-sore of cattle in India (1935). The occurrence of *Stephanofilaria* (*sp*.) causing ear-sore in the buffalo, is interesting.

A question may be asked whether the parasite is a direct etiological agent causing ear lesions. Although transmission experiments, here as well as elsewhere, have not been successful so far in all the skin affections caused by parasites of the Stephanofilaria species, other evidences substantially establish the disease to be of nematode origin. The constant presence of the parasite in the lesions, the distribution of the worms in the affected parts and type of cellular reaction set up in the skin lesion, clearly suggest the cause of the disease.

Ear-sore in buffaloes is reported to occur in Bihar for some years past and at times in an epidemic form. While investigating the condition, Kapur [1940] has stated that in one case three microfilaria-like objects were seen in the pus obtained from an infected ear. The report on the material sent to the Indian Veterinary Research Institute, Mukteswar, for examination, states that a considerable number of microfilaria were seen in section burrowing through and below the epithelial papillae of the skin. Presumably no adult parasite was obtained from the lesions. It seems evident from the report that the condition as reported in Bihar, is similar to some extent, to the disease described in this paper.

Clinical observation indicate that some parallelism exists between ear-sore of buffaloes and hump-sore of cattle in certain respects. The nature of skin lesions in quescent and active stages, the seasonal incidence, the tendency to become chronic, the presence of apparently identical parasites, their absence in general circulation and finally, the response to tartar emetic treatment, point to similar pathological process though in different species of animal and places of predilection.

SUMMARY

Details are given of investigation of Stephanofilariasis among buffaloes in Assam and of its occurrence as a specific cutaneous microfilariasis of the ear. The disease usually occurs sporadically but at times, it prevails in the form of an epidemic. Full particulars of its seasonal occurrence and localities affected, are given.

Symptoms, course, means of control and treatment are described. The disease is generally confined to buffaloes. It is very rarely fatal though the incidence of affected animals in an outbreak varies from 20 to 50 per cent.

The causal parasite, both microfilaria and adult nematode, has been recovered from the lesion of ear-sore. The morphological characters of the parasite have been found apparently identicals with those of *Stephanofilaria assamensis* [Pande 1936], causing hump-sore of cattle in India. The findings have been confirmed by Mukteswar Institute and by Officer-in-charge, Helminthiasis Scheme, Lucknow University.

Skin affections of cattle in India and elsewhere, caused by *Stephanofilaria sp.*, are cited. The occurrence of *Stephanofilaria sp.* in buffalo—a different species of animal causing ear-sore, is recorded.

Observations on certain identical features of hump-sore of cattle and ear-sore of buffaloes are discussed.

The work has been carried out under a scheme of research financed by the Indian Council of Agricultural Research, India.

ACKNOWLEDGMENTS

I wish to express my sincere thanks to Dr. G. S. Thapar, Officer-in-charge, Helminthiasis Scheme, Indian Council of Agricultural Research, Lucknow University, Lucknow, and to Dr. S. C. A. Datta, Officer-in-charge, Veterinary Zoology Section, Indian Veterinary Research Institute, Izatnagar, for examination of specimens. I wish to place on record acknowledgment of help and facility received from Rai Bahadur S. C. Ghose and from Mr. A. K. Mitra, Director of Veterinary Department, Assam. To Mr. Nag Chowdhury, Veterinary Assistant Surgeon, Patharkandi, my thanks are due for his assistance in the field work.

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PREPARATION OF VEGETABLE RENNET FROM *WITHANIA COAGULANS*, DUNAL

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(Received for publication on 18 June 1948)

An enzyme which can be used in the place of animal rennet in the preparation of cheese will prove very useful taking into consideration the particular food habits of our population. The berries of *Withania coagulans*, Dunal, are known to contain an active material useful for this purpose [Lea, 1883-84]. Recently Kothavalla and Khubchandani [1940] used a saline extract of *Withania coagulans* for preparing cheeses. These authors used a temperature of 105°F. for coagulation. Some difficulty in preparing hard cheeses of satisfactory quality was experienced as they developed open texture. Narain and Singh [1943] have described the properties of alcohol precipitated enzyme and also used this material for making Cheddar cheese.

Since Kothavalla and Khubchandani carried out their work at this Institute, further experiments have been in progress with the object of isolating an enzyme which can be used in place of animal rennet, under the accepted working conditions. The results of this work are described here.

Experimental

(*a*) *Preparation of extract.* Preliminary trials were carried out to find out where the enzyme material is stored in the plant. Leaves and stems of the plant and seeds from the berries were found to be devoid of all enzyme material. The pulp in the berries was the most active source of milk clotting enzyme, and the aqueous extract of the outer husk of the berries had about a third of the activity of the pulp. For experimental work, an extract of the whole berries was used as it was very tedious to remove the seeds embedded in the pulp and the weight of husk in relation to the pulp was very small. For extracting the enzyme, various materials in different concentrations were tried, viz., water, NaCl (0·3 to 10 per cent), Na_2CO_3 (5 per cent), glycerine, alcohol (5 per cent), HCl (1-5 per cent), CaO (1 per cent) and $CaCl_2$ (0·3-2 per cent). Distilled water and 1 per cent NaCl proved most suitable.

The extraction was carried out as follows : 1,000 gm. of berries were ground with about 750 ml. of water to a thin paste. This was centrifuged when a considerable amount of insoluble material settle down. The top liquid was opaque brown. The residue was washed twice with water and separated in the manner described. The total quantity of water used was 850 ml. and the yield after centrifuging was about 700 ml. The extract was quite stable at room temperature even without any antiseptics. On storage, both in cold and at room temperature, some insoluble material deposited from the extract. The precipitate formed was completely inactive and the removal of this deposit by centrifuging had no effect on the activity of the supernatant liquid. Various preservatives were added to see if these would prevent the formation of the precipitate. The substance tested included borax (0·5 per cent), boric acid (1 per cent), mustard oil (1 per cent), glycerine (5-10 per cent), NaCl (5-10 per cent), alcohol (5 per cent) toluence and combinations of some of these. None of these preservatives was able to prevent the formation of the precipitate.

(*b*) *Determination of activity.* Activity was determined using milk substrate at a temperature of 37°C. Fresh milk, and milk adjusted to an acidity of 0·2 per cent lactic acid by adding a suitable quantity of 0·25 N pure lactic acid solution, were used. This acidity was adopted as this is the acidity in milk normally used for cheese making. One ml. of rennet solution under test was added to 50 ml. of milk and time of clotting when first flakes began to appear was noted in seconds. Each result given in the subsequent Tables represents the mean of two observations.

At a later stage it was found that $CaCl_2$ enhances the activity of the enzyme and so milk was fortified with Ca by adding 2 ml. of 12·33 per cent $CaCl_2$ solution to 98 ml. of milk.

(c) *Concentration of the enzyme*

(i) *Concentration under vacuum.* Attempts were made to concentrate the enzyme by distilling off water under vacuum at 40-45°C. A heavy froth was produced during distillation. In the end a thick pasty mass was obtained resembling molasses in appearance. Attempts were made to dry this material over sulphuric acid under vacuum without success. Eight hundred and fifty grammes of the aqueous extract gave nearly 275 gm. of the concentrate after vacuum distillation and drying. This method was abandoned as it did not promise to give useful results.

(ii) *Precipitation with acids and alkalies.* When the aqueous extract was treated with dilute H_2SO_4, HCl, NH_4OH and NaOH, no precipitation occurred.

(iii) *Precipitation with salts.* Sodium chloride, ammonium sulphate and magnesium sulphate were used for this purpose 1,000 ml. of aqueous extract required 350 gm. of NaCl for complete precipitation of the precipitable matter. Some precipitate was obtained at 20·8 and 38·4 per cent ammonium sulphate but the bulk of the precipitate was at 39·0 per cent concentration. Magnesium sulphate gave precipitates at 22 per cent and 53 per cent concentrations. With each of the above precipitants, when complete precipitation had occurred the clear supernatant liquid was decanted off and the solution containing the precipitate was dialyzed after adding some toluence. When the dialysate was free from the added salt, the bag containing the material was removed and the contents transferred to a measuring flask of 250 ml. capacity and made up to the mark. During the process of dialyzing some precipitate was formed which did not go in solution and which was inactive. For the determination of activity, the whole suspension was used in each case. The results showed that in no case was a highly active material obtained. As this method of precipitation was likely to prove tedious under commercial conditions, it was not perused further.

(iv) *Precipitation with organic solvents*

(a) *Precipitation with alcohol.* Each 100 ml. of water extract required 250 ml. of absolute alcohol for precipitation of the active enzyme. First signs of a precipitate occurred after 160 ml. of alcohol were added. By this method about 9 gm. of dry powder was obtained from 100 ml. of aqueous extract. Larger yields could be obtained by adding more alcohol to the supernatant liquid but the precipitate formed was quite inactive. The enzyme powder was not completely soluble in water and on keeping, insoluble matter settled down. The alcohol precipitate had good milk clotting properties as shown in the following Table.

4 per cent solution of	Clotting time of milk adjusted to 0·2 per cent lactic acid
Sample I	61
Sample II	63
Animal Rennet	65

The results show that it is possible to get an active enzyme by this treatment. Similar results have been reported by Narain and Singh [1943].

(b) *Precipitation with acetone.* By trials it was found that the optimum amount of acetone to be added was two volumes to one volume of aqueous extract. Aqueous solution of *Withania coagulans* of different strength were made and precipitated with acetone. It was observed that the enzyme precipitated from dilute solutions was more active. Extraction of *Withania coagulans* berries with a 5 per cent $CaCl_2$ solution and adding acetone to this extract gave a completely inactive product.

Attempts were also made to first precipitate the enzyme with NaCl. $(NH_4)_2 SO_4$ and $MgSO_4$, dissolve the salt free precipitates in H_2O and then again precipitate with acetone. The precipitates so obtained did not prove more active than the enzyme obtained by precipitation from the original water extract. The final process evolved for the preparation of the enzyme by acetone precipitation was as follows: 1,000 gm. of *Withania coagulans* berries were extracted with two volumes of H_2O and centrifuged when a clear extract was obtained. One volume of this extract was treated with two volumes of acetone with stirring. After about 15 minutes, the supernatant liquid was decanted off and the precipitate washed two to three times with a little acetone. It was then dried over H_2SO_4 under vacuum. 1,000 ml. of extract gave 10-15 gm. of a grayish powder, which was quite stable even when stored at room temperature. To enhance the activity of this concentrate, one part of the dry enzyme was mixed with two and a half times its weight of anhydrous $CaCl_2$. The activity of the material obtained as above can be increased by dissolving it in water and reprecipitating with acetone as shown below, but for studies described here only the once precipitated enzyme was used.

Clotting time with 10 per cent Hansen's liquid rennet	40
Clotting time with single ppted. enzyme (5 per cent soln. in 12·33 per cent $CaCl_2$) .	52
Clotting time with thrice ppted. enzyme (5 per cent soln. in 12·33 per cent $CaCl_2$) .	44

The activity of the once precipitated enzyme was compared under identical conditions with Hansen's liquid and powder rennets as shown in Table I. Vegetable rennet was dissolved in 12·33 per cent $CaCl_2$. The activities were compared at 37°C. and 45°C., the latter as shown later, was the optimum temperature for vegetable rennet.

TABLE I

Comparative activity of vegetable and animal rennets (expressed as time taken to curdle 50 ml. of milk by 1 ml. of rennet solution)

At 37°C.			At 45°C.			
Hansen's liquid rennet 10 per cent	Vegetable rennet 5 per cent	Hansen's rennet powder 1 per cent	Hansen's liquid rennet 10 per cent	Vegetable rennet 5 per cent	Animal rennet powder 1 per cent	Animal rennet powder 1·5 per cent
20	34	20	16	7	13	11

The results show that at its optimum temperature, the enzyme from *Withania coagulans* is very active and compares favourably with the imported materials. On this basis it is estimated that one part of the vegetable rennet will coagulate 90,800 parts of milk in nearly 30 minutes.

(*d*) *Properties of Withania coagulans enzyme*

(i) *Optimum temperature*: A 5 per cent solution of the enzyme in 12·33 per cent $CaCl_2$ was used. Side by side, trials were also made with a 10 per cent solution of Hansen's liquid rennet for comparison. The results are shown in Table II.

TABLE II

Optimum Temperature of Withania coagulans *enzyme*

	Temperature °C.								
	37	40	45	50	55	60	65	70	75
	Coagulation time in seconds								
Vegetable rennet . . .	68	50	21	21	23	22	23	25	60
Animal rennet . . .	28	24	23	19	20	21	25	90	..

The optimum temperature of the vegetable rennet was between 45-65°C. The optimum temperature of the animal product also lay within the same range. At temperature below 45°C. the activity of vegetable rennet was markedly affected and at 37°C. it was only about a third of the activity at 45°C. The activity of the animal rennet was affected comparatively to a small degree by changes in temperatures within the range indicated.

(ii) *Effect of* pH. Milk was adjusted to different acidities and the *p*H determined using quinhydrone electrode. The enzyme solutions were of the strength indicated before.

TABLE III

Optimum pH of Withania coagulans *enzyme*

Acidity of (per cent lactic)	Milk	Trial No. 1		Trial No. 2		
	*p*H	Vegetable rennet	Animal rennet	*p*H	Vegetable rennet	Animal rennet
		(Coagulation time in seconds)			(Coagulation time in seconds)	
0·12	6·80	100	100	6·60	93	90
0·14	6·68	99	79	6·48	85	65
0·16	6·56	75	47	6·35	75	47
0·18	6·46	67	38	6·22	67	40
0·20	6·38	62	30	6·11	62	35
0·22	6·24	55	25	6·01	58	32

With the increase in acidity, the time of coagulation was found to decrease both with vegetable and animal rennets. This increase in activity was more in the case of animal product.

(iii) *Effect of heat.* 10 ml. of solutions of vegetable and animal enzymes were placed in a test tube and the tube immersed in a beaker of water maintained at a particular temperature. After the solution had attained the required temperature, it was maintained at that temperature for 5 minutes. The results are shown in Table IV.

TABLE IV

Effect of heat on rennets

——	Temperature °C.					
	37°	40°	50°	60°	70°	80°
	Coagulation time in seconds					
Vegetable rennet	60	58	60	73	152	Over 600
Animal rennet	32	32	32	51	Over 600	"

The results show that both the vegetable and animal rennets are sensitive to heat. Heating over 50°C. even for short duration, effects the activity of the solutions of these rennets adversely.

(iv) *Effect of using boiled milk.* Milk was brought to first boil and then allowed to cool to 37°C. As substrates milk and milk containing $CaCl_2$ were used. The results are given in Table V.

TABLE V

Effect of using boiled milk on the activity of Withania coagulans *enzyme*

Milk used	10 per cent animal rennet	5 per cent vegetable rennet in $CaCl_2$
	(Coagulation time in seconds)	
Cow milk raw	30	70
Cow milk boiled	39	97
Cow milk boiled-fortified with Ca	..	84
Buffalo milk raw	26	47
Buffalo milk boiled	32	74
Buffalo milk boiled-fortified with Ca	27	62

It was found that heated milk takes longer time to coagulate. Action of vegetable rennet was much slower than that of animal rennet. Addition of $CaCl_2$ compensates to a large measure the effect of heat. The figures for the relative time for the coagulation of cow and buffalo milk are interesting. Buffalo milk on an average was found to take less time for coagulation either with animal or vegetable rennets.

(v) *Effect of various absorbents.* A 5 per cent aqueous solution of acetone precipitated enzyme was used. 50 ml. of this solution were treated at room temperature with 5 per cent of the following :—

(*a*) Animal charcoal (+++++++).

(*b*) Rice husk charcoal (+++++++).

(*c*) Fuller's earth (++++++++++).

(*d*) Kaolin (+).

(*e*) Aluminium oxide (+++++).

(*f*) Calcium carbonate (++++).

(*g*) Calcium hydroxide (++++++++++).

(*h*) Kiesulghar (++).

After keeping for about 30 minutes with occasional stirring, the solutions were filtered through a filter paper. The degree of clarification obtained was noted visually and is indicated by (+) signs. Activities of the filtrates were tested using milk acidified to 0·2 per cent lactic acid and fortified with $CaCl_2$. The results are shown in Table VI.

TABLE VI

Activity of vegetable rennet solutions treated with absorbants

Original solution	Vegetable rennet solution treated with 5 per cent of							
	Animal charcoal	Rice husk charcoal	Fuller's earth	Kaolin	Aluminium oxide	Calcium carbonate	Calcium hydroxide	Kiesulghur
	(Coagulation time in seconds)							
149	231	152	305	243	166	160	Over 1,200	229

It was found that $Ca(OH)_2$ wholly destroyed the activity of the filtrate. Similar results were obtained even with 1 per cent $Ca(OH)_2$. Fuller's earth, Kiesulghar, Kaolin and animal charcoal, calcium carbonate and aluminium oxide and rice husk charcoal decreased the activity in the order mentioned. Attempts were made to extract the enzyme from these absorbents with various media without success. Some of the absorbents were alkaline and so the filtrate obtained was acidified and then the activity tested. Still the solutions did not show enhanced activity.

(vi) *Toxicity of* Withania coagulans *extract.* This was tested with rats. Four young male rats were fed 4 ml. of a 10 per cent extract of the berries for a period of ten weeks. An equal number of animals were kept as controls. The weight of animals was recorded. No difference was found in the growth rate of control and experimental groups of animals. It was concluded that the extract of the berries was non-toxic.

(vii) *Effect of different salts and sunlight.* That calcium chloride considerably enhances the activity of the *Withania coagulans* enzyme has been indicated previously. Effect of other inorganic salts was investigated. Amongst the salts tried were $ZnCl_2$ (0·004 per cent), KI (0·005 per cent), Cu_2O (0·04 per cent) and bleaching powder (0·04 per cent). The salt to be tested was added to milk. Vegetable rennet solution was made in 12·33 per cent $CaCl_2$. The results are given in Table VII.

TABLE VII

Effect of salts on the activity of vegetable rennet

5 per cent Vegetable rennet in water	5 per cent vegetable rennet in 12·33 per cent $CaCl_2$ solution				
	——	Salt added to milk			
		$ZnCl_2$ (0·004 per cent)	Cu_2O (0·04 per cent)	KI (0·005 per cent)	Bleaching powder (0·04 per cent)
		Coagulation time in seconds			
210	78	194	107	84	88

It is seen that none of the substances tried except $CaCl_2$ enhanced the activity. In fact they all produced a slight inhibitory effect.

Vegetable rennet solution in H_2O and in $CaCl_2$ were exposed to strong sunlight and the activity determined at intervals. Strong sunlight was found not to effect the activity of vegetable rennet. Narain and Singh [1942] also state that radiations of different wave-lengths have no influence on the activity of the enzyme.

(viii) *Preparation of cheese.* Both soft (*Surti panir*) and hard (Chaddar) cheeses were prepared from various concentrates of *Withania coagulans* obtained from time to time. As promising results were obtained in the beginning with the vegetable rennet, no attempt was made to deviate from the standard technique followed with animal rennet. A few difficulties were encountered in the beginning but these were gradually overcome. It will, however, be interesting to record some of the main defects encountered in the beginning for information.

(a) *Soft cheese.* The texture was very crumbly. Cheese when kept dipped in whey gradually became thinned at the edges.

(b) *Hard cheese.* Cheese developed open texture on keeping. There was a bitter and biting taste which made the product unpalatable. Cheese was soft and would not cut clean.

As better quality rennet material was obtained, these difficulties were gradually overcome and it was possible to make as good a soft cheese with vegetable rennet as with the product obtained from animal source. A summary of a comparative operation for the manufacture of *Surti panir* is shown in Table VIII.

The method of preparation of *Surti panir* has been described in detail by Kothavalla and Verma [1942] and was followed in the present investigation. For dipping vegetable rennet cheese, various media, including animal rennet whey, 1·5 per cent salt solution, 0·05 per cent lactic acid solution in 1·5 per cent NaCl, were tried and in the end it was found that vegetable rennet whey obtained during the operation was the most satisfactory.

TABLE VIII

Preparation of Surti panir *with animal and vegetable rennets*

Particulars of operation	Hansen's liquid rennet	Vegetable rennet 5 per cent Soln. in 12·33 per cent $CaCl_2$
Milk taken (6·5 per cent fat)	2 lbs.	2 lbs.
Temperature of milk	95°F.	95°F.
Starter added	1 oz.	1 oz.
Acidity of starter	0·65 per cent lactic	0·65 per cent lactic.
Amount of rennet added	4 drops	24 drops.
Time rennet added	9-30 a.m.	9-35 a.m.
Time cut	10-40 a.m.	10-45 a.m.
Time first turned	11-45 a.m.	11-50 a.m.
Time dipped in whey	4-30 p.m.	4-30 p.m.
Fat per cent of whey	0·50 per cent	0·50 per cent.
Remarks: Colour	White	White.
Texture	Good	Good.
Taste	Good	Good.

On the whole *Surti panir* prepared by animal and vegetable rennets were indistinguishable.

Trials were also carried out to prepare Chaddar cheese with vegetable rennet. By trials it was observed that longer time is required for the ripening of Chadder cheese made with *Withania coagulans* and it should possibly be extended to six months. As the time of ripening increased, the bitter taste diminished. It has, however, not been possible to remedy this defect completely. Further work is in progress to purify the enzyme and it is hoped that it will be then possible to get over this difficulty.

Summary

1. Seeds, leaves and stems of *Withania coagulans* show no milk clotting property. The pulp of the berries was the most active source of the enzyme. A little activity was also shown by the outer husk.

2. For the preparation of an enzyme concentrate, the enzyme was first extracted with water and then precipitated with two volumes of acetone. The activity of the acetone precipitated enzyme compared well with that of Hansen's liquid and powder rennets.

3. The optimum temperature of *Withania coagulans* enzyme lay between 45-65°C. At higher temperatures than 70°C., the activity of the enzyme decreased rapidly and it was completely inactive at 80°C.

4. The activity of the enzyme increased with an increase in the activity of milk.

5. Boiled milk took a longer time to clot but the lost activity could be restored to a large measure by adding calcium chloride.

6. *Withania coagulans* extract is non-toxic.

7. Different absorbents decrease the activity to different degrees.

8. Soft cheese of a quality indistinguishable from that made with animal rennet was prepared.

Acknowledgments

The authors wish to express their thanks to the Indian Council of Agricultural Research for giving a special grant for this work. They are also grateful to Dr K. C. Sen, Director of Dairy Research and Dr Zal R. Kothavalla, Dairy Development Adviser for their helpful suggestions and criticism.

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UTILIZATION OF WHEY—A MILK WASTE IN THE PRODUCTION OF '*CHHANA*'—FOR SUPPLY OF CALCIUM TO THE POOR RICE DIET

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(Received for publication on 14 May 1948)

CALCIUM deficiency is one of the fundamental defects of the poor rice-eaters' diet in India and attempts have been made in this laboratory to explore some cheaper sources of calcium to be supplemented with the poor rice diet Basu *et al* [1942]. It has been shown, that, the lime taken up in the process of chewing betal leaves and bones of small fish, can make up the calcium deficiency of the poor rice diet. Further attempts have been made to find out some other cheaper source of nutritionally available calcium.

In India, a large amount of milk is daily used in the production of coagulated milk or '*chhana*' which is employed in the manufacture of sweets consumed by the richer section of the population. The whole amount of whey containing valuable nutritive materials as lactose, calcium, protein etc., is thrown away every day as waste. In a province like Bengal about 34,000 md., i.e., 1,200 tons of whey is thrown away daily as such. Our aim was, to see whether this whey which is thrown away as waste can be utilized as food supplement for supply of calcium to the poor rice-eaters' diet of India.

Experiment and results

Metabolic experiment was carried out on two healthy adult human subjects of 42 and 44 kg. body weight and 11 adult rats of 162 to 213 gms. body weight who were kept on the poor rice diet of the following composition.

Table I

Composition of the diet

Name of the foodstuff	Weight in grammes
Rice	550
Pulse	100
Vegetables :—	
Potato	100
Green banana	50
Cabbage	50
Oil-Groundnut	30

Daily whey supplement
(*a*) For human subject – 200 c.c.
(*b*) For rats – 6 c.c.

In case of the human experiments, the above quantity of the diet was given daily to the subjects and in case of rat experiments, the basal diet was prepared according to the above composition after drying all the ingredients and each rat was given 10 to 15 gms. of the diet according to the requirement. Whey supplementation was begun after a basal period of six days on the rice diet and this was continued for further period of six days. In both basal and supplementation periods the collection of urine and faeces was made on the last three days of the period and their calcium was estimated according to the method adopted in the previous communication from this laboratory [Basu *et al* 1942].

In case of human experiments, the whey supplement was administered along with *'dal'* and vegetables after cooking them with it, but in case of rat experiment, the whole diet was cooked with whey. Whey was daily collected from the market and its calcium content was found to range between 0·62 to 1·3 mg. per c.c. The results of metabolic experiments with human subject and rats are presented in Table II from which it is observed that both the subjects J. C. D. and K. N. S. maintained negative calcium balance when living on poor vegetarian rice diet. The average intake and balance of calcium on rice diet was found to be 237·1 mgm. and—74·3 mgm. respectively. The administration of 200 c.c. of whey daily to the diet furnished on average 133·4 mgm. of extra calcium to the diet and improved the negative balances of the two subjects J. C. D. and K. N. S. from —127·3 to +4·4 mgm. and from —21·2 to +83·3 mgm. respectively. The percentage utilization of extra calcium of whey supplement was calculated to be 94·5 and 82·5 respectively.

In adult rat experiments the mean daily calcium intake and balance were found to be 9·01 and —0·7 mgm. respectively and the supplementation of 6 c.c. of whey to the diet supplied on average daily 5·73 mgm. of extra calcium and improved the balance from —0·7 to +4·1 mgm. In this case the average percentage utilization was found to be 83·7.

Although the whey is produced as waste from milk, the utilization value of its calcium determined in the present work, is found to be considerably higher than that of milk. By a series of balance studies on children and adults Kinsman *et al* [1939], Steggerda and Mitchell [1939] and Brieter *et al* [1941] have observed the utilization value of milk and calcium between 12 to 29 per cent.

TABLE II

The calcium utilization of whey by adult human subjects and rats. Each period consisted of three days and the figures indicate the daily averages for the three days collection. The periods which are not indicated in the Table should be regarded as preliminary periods

Human experiment

Experimental subject	Experimental diet	Supplement	Period	Dietary calcium mg. per day	Total output of calcium (urinary and faecal) mg. per day	Balance of calcium mg. per day	Per cent utilization of whey calcium
J.C.D. Body wt. –42 Kg. Age –22 years.	Rice diet	*Nil*	II	248	375·3	–127·3	–
	Rice diet	200 c.c. whey	IV	248+139·3 =387·3	382·9	+4·4	94·5
K.N.S. Body wt. –46 Kg. Age –25 years	Rice diet	*Nil*	II	226·2	247·4	—21·2	–
	Rice diet	200 c.c. whey	IV	226·2+127·5 =353·7	269·9	+83·8	82·5

Rat experiment

Average body wt. of the rats – 187 gm.	Rice diet	*Nil*	II	9·01	9·71	—0·7	—
	Rice diet	6 c.c. whey	IV	9·01+5·73 =14·74	10·61	+4·1	83·7

The higher utilization value of whey calcium above 80 per cent observed here in both human and rat experiment may be due to the presence of sufficient quantity of lactic acid in it.

It has previously been pointed out that about 1,200 tons of whey is daily thrown away as waste in Bengal. Taking the average calcium content of whey as 0·7 mg. per c.c., the net utilizable calcium wasted away daily along with whey is approximately 9 tons. If 200 c.c. of whey is given as food supplement to each adult, the above quantity of whey can make up the deficiency of 5·5 million people of Bengal. So for practical nutrition, whey thrown away as waste in the production of sweets for rich section of the people may be used as a calcium supplement for the poor and the present work opens a new avenue for the fortification of poor Indian diets.

Summary

Administration of 200 c.c. of whey with the poor rice diet would supply about 137 mg. of extra calcium and make up the deficiency of the rice diet with regard to this essential element. By both, rat and human experiments the percentage utilization of the whey, calcium was found between 80 to 90 per cent.

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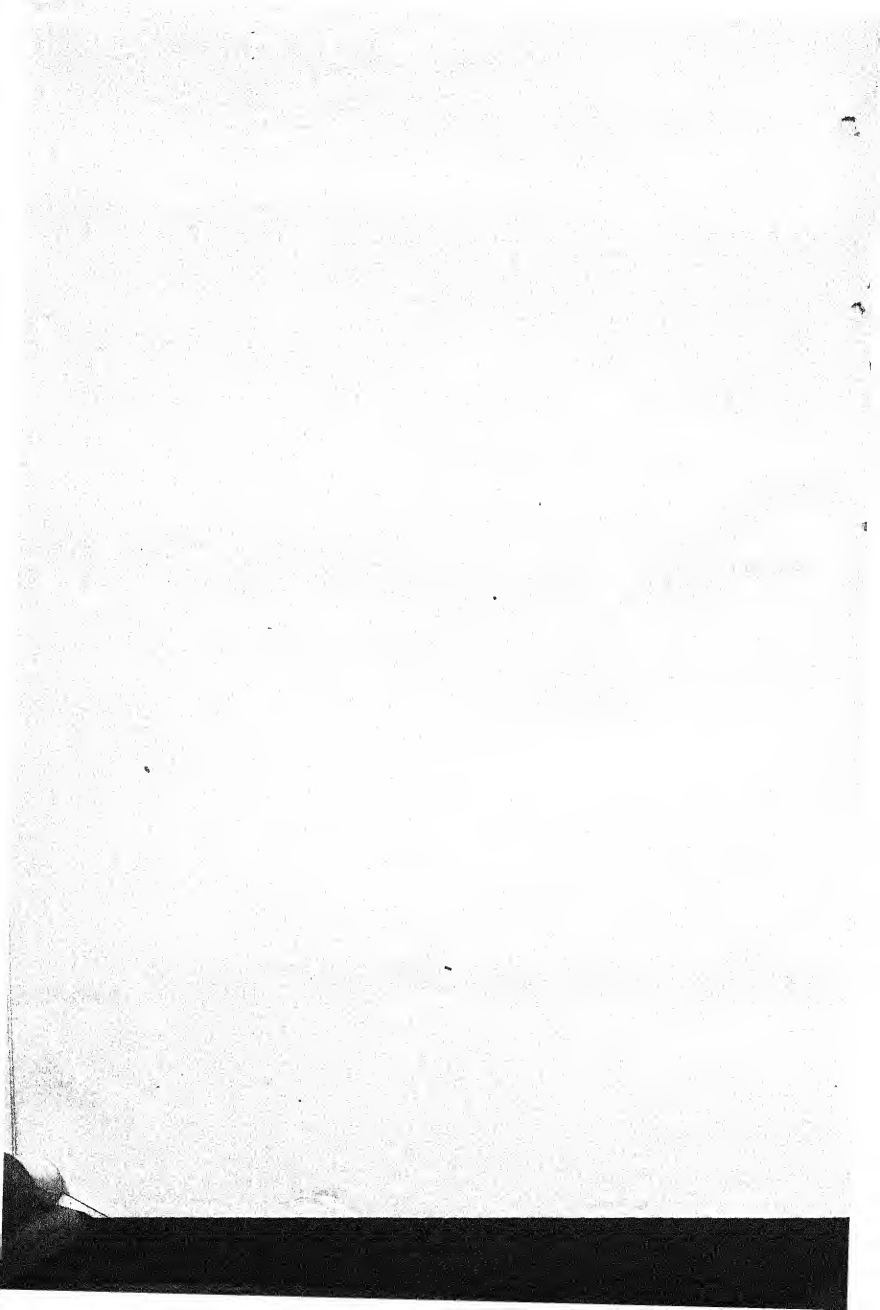

KHAKHRA DHAND IN THE PUNJAB AS PROBABLE BREEDING GROUND AND NURSERY FOR THE THAILA FISH, *CATLA CATLA* (HAMILTON)

By UDE SINGH RAI, M.Sc., Ph.D., Warden of Fisheries, East Punjab, Palampur

(Received for publication on 16 April 1947)

(With one text figure)

IN a recent Symposium on the 'Factors Influencing the Spawning of Indian carps' ; several fishery scientists, namely, Hora, Mookerjee, Hamid Khan, Amjad Husain, Das and Das Gupta, Sundara Raj, Moses, Dasen, Langdale Smith, Mazumdar and Nazir Ahmad, participated in the discussion. The general conclusions arrived at were thus summarized by Hora in his concluding remarks :

'The flooded condition of a river or a tank is the primary factor that is responsible for the spawning of Indian carps. It has also been brought out that intensive flooding capable of inundating vast shallow areas, which form the spawning grounds of fish, could only induce the spawning of Indian Carps. By creating floods artificially, which should be of sufficient intensity so as to inundate spawning grounds, at Radhanagar, Midnapore and at Satkania, Chittagong, it has been possible to induce spawning and reversely when low floods in the Punjab did not inundate spawning grounds, the fish refused to come out for spawning though they were sexually mature. The high *p*H and oxygen content value of water are a necessary corollary to floods but have no independent value in inducing spawning. Both *p*H value and oxygen tension could be artificially controlled, but it will generally be conceded that these factors by themselves have no value unless the spawning grounds are covered for some time, so as to enable fish to induce in amorous play, liberate eggs and give sufficient warmth for the helpless larvae to pass a few days of their life in the seclusion and rich pasturage characteristic of shallow waters.

One other factor of importance which seems to affect spawning independently of floods, is the temperature. It has been observed in the Pubjab that as soon as the temperature rose from 76°F. to 96°F., the fish, which had responded to the floods, deserted the spawning grounds. Similarly, Das and Das Gupta found that in the Kukuti Bundh, Midnapore, so long as the temperature was above 84°F. two batches of fish were induced to spawn successfully but the third batch refused to spawn as the temperature had fallen to 81°F. Mr Langdale Smith's observations on the breeding of Mahseer and from our knowledge of the factors influencing the spawning of European Carp, it is also clear that temperature is a factor of considerable importance.'

From the observations recorded below regarding the probable breeding of *Catla* in the Khakhra 'Dhand', it would appear that flood is undoubtably a primary factor though it is not caused by the monsoon rains but by the melting of snow. Unfortunately no regular records of temperature were maintained but from the analysis of total salts and *p*H given here it is clear that waters of the 'Dhand' undergo considerable variation in these respects at the time of the breeding of the fish.

THE KHAKHRA 'DHAND'

The Khakhra 'Dhand' is situated in the course of the Khakhra tributary of the Sutlej river and is an extensive low, marshy area, situated in the jurisdiction of the village Bhangala, near Valtoha Railway station, in the District of Amritsar. It is one and a half miles long and at least quarter of a mile broad during the flood season, but during the dry season its breadth shrinks and is not more than one hundred yards. It is connected with the river Sutlej by a perennial channel which is about a mile in length, and is fed by the Khakhra 'Dhand'.

1. *Proc. Nat. Inst. Sci.* India, IX, pp. 303-330 (1943)

Khakhra tributary in which the Khakhra 'Dhand' lies, is the old bed of the river Beas. The rivers Sutlej and Beas at one time flowed parallel to each other for a considerable distance in the districts of Amritsar, Lahore and Montgomery. As the river Beas now falls into river Sutlej at 'Hari-ke-Pattan', about twenty miles above the confluence of the channel of the Khakhra tributary into the river Sutlej, the Khakhra tributary serves as a drain of the low-lying area between the river Sutlej and the old bed of the river Beas.

The level of the water in the Khakhra 'dhand' and the channel of the Khakhra tributary rises, as the level of the water in the river Sutlej gets higher due to the melting of snow, ultimately flooding the fields lying on either side of the channel and some part of the 'dhand'.

Apart from the Khakhra 'dhand', a few more big pools are found in the Khakhra tributary in its upper reaches. But, they are not appreciably affected by the rise of level in the river Sutlej during the floods.

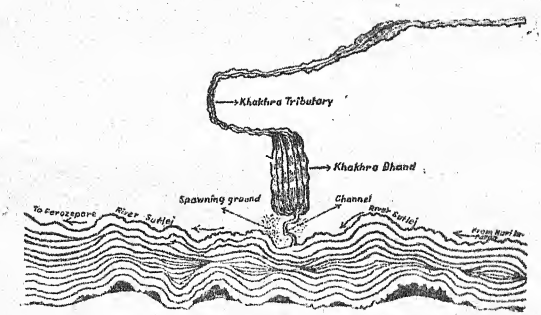

FIG. 1. Khakhra Dhand the spawning ground for *Catla* and other carps

Evidence of Catla *breeding in the 'Dhand'*

The actual courtship, wed-lock and spawning have not been observed, but the following facts indicate fairly clearly that the fish breeds in the 'dhand'.

1. Large-sized *Catla*, presumably sexually mature, have been observed entering the 'Dhands' from the river Sutlej in the month of June.

2. Spent *Catla* have been caught while going down to the river in September.

3. Sixty per cent of the fish population of the 'dhand' consists of young *Catla*.

4. Flooding of the river and in consequence of the channel and the 'dhand' takes place in June and July owing to the melting of the snow, but independent of monsoon rains. As floods due to melting of snow occur every year, the spawning also occurs in the 'Dhand' every year.

5. The sides of the channel as well as of the 'dhand' provide suitable spawning beds when covered with flood water.

6. The temperature never rises above 90°F., and is therefore, suitable for *Catla* spawning.

Composition of 'Dhand' water before and during flooding

TABLE I

Results of water-analysis of Khakhra 'dhand' per 100,000 parts, during June and July, 1946

Date	Place of collection	Total salts	Carbonates	Bi-carbonates	Chlorides	Sulphates	Calcium	Dissolved oxygen per litre	Required oxygen	pH	Phosphates	Nitrates
12-6-46	Surface bank	204·25	23·32	152·04	22·8	6·27	Traces	2·657 cc.	3·59 gms.	8·36	Traces	0·0625 mgms.
	Bottom bank	207·63	20·14	157·08	23·4	7·98	Traces	2·279 cc.	3·66 gms.	8·38	Traces	0·04 mgms.
	Surface middle	212·73	19·08	156·24	21·6	16·7	Traces	2·519 cc.	3·16 gms.	8·47	Traces	0·0125 mgms.
	Bottom middle	220·52	21·2	168·00	28·08	9·38	Traces	2·183 cc.	2·92 gms.	8·47	Traces	0·015 mgms.
21-6-46	Surface Bank	41·37	2·12	20·16	3·51	13·4	2·96	4·100 cc.	0·315 gms.	9·06	Traces	0·0325 mgms.
	Bottom bank	36·89	8·48	18·45	3·51	9·2	1·92	3·543 cc.	0·825 gms.	9·04	Traces	0·0375 mgms.
	Surface middle	29·30	6·36	11·70	5·85	5·17	0·96	3·768 cc.	0·230 gms.	9·05	Traces	0·02 mgms.
	Bottom middle	31·29	12·72	10·08	5·85	4·87	1·68	3·457 cc.	0·280 gms.	9·03	Traces	0·0325 mgms.
19-7-46	Surface bank	—	—	—	—	—	—	3·756 cc.	0·660 gms.	7·0	—	—
	Bottom bank	—	—	—	—	—	—	3·617 cc.	0·640 gms.	7·0	—	—
	Surface middle	—	—	—	—	—	—	3·798 cc.	0·720 gms.	7·0	—	—
	Bottom middle	—	—	—	—	—	—	3·788 cc.	0·70 gms.	7·0	—	—
28-7-46	Surface bank	31·89	*Nil*	21·84	5·26	5·79	1·56	3·150 cc.	0·515 gms.	7·0	Traces	0·126 mgms.
	Bottom middle	31·95	*Nil*	17·64	4·12	10·46	1·8	3·045 cc.	0·55 gms.	7·0	Traces	0·124 mgms.
	Surface middle	30·25	*Nil*	15·12	4·68	10·15	1·43	3·642 cc.	0·58 gms.	7·0	Traces	0·125 mgms.
	Bottom middle	31·25	*Nil*	16·18	5·26	9·15	1·56	3·349 cc.	0·44 gms.	7·0	Traces	0·126 mgms.

TABLE II

Variation in amount of total salts in 'Khakhra dhand' water, from October, 1945 to September 1946

Month	Total salts per 100,000 parts
October, 1945	67·71 parts to 97·25 parts
November, 1945	78·20 parts to 100·2 parts
December, 1945	81·05 parts to 83·2 parts
January, 1946	82·22 parts to 94·20 parts
February, 1946	88·42 parts to 89·25 parts
March, 1946	100·45 parts to 101·23 parts
April, 1946	127·92 parts to 170·25 parts
May, 1946	145·05 parts to 174·27 parts
12 June, 1946	204·25 parts to 220·52 parts
27 June, 1946	29·30 parts to 41·37 parts
28 July, 1946	30·25 parts to 31·95 parts
31 July, 1946	29·79 parts to 31·05 parts
10 August, 1946	25·12 parts to 29·09 parts
21 August, 1946	75·26 parts to 78·20 parts
31 August, 1946	71·01 parts to 76·18 parts
14 September, 1946	88·56 parts to 90·25 parts
27 September, 1946	114·28 parts to 115·25 parts

From the Table above it is observed that—1. The presence of total salts in the 'dhand' water continued to increase from October, 1945 to June, 1946. Ultimately in June 1946, the increase of total salts in the 'dhand' water was found to be ten times to that in the river-water, as is evident from the Table II, given above.

2. River-water entered the 'dhand' during the second fort-night of June, 1946 and diluted the 'dhand' water, as is clear from the figures given in Table II.

3. Analysis of 'dhand' water revealed that its *p*H value was 9·9 before the spawning, and the *p*H value was 7·0 after the spawning had taken place due to the dilution caused by the inrush of river-water into the 'dhand', as is evident from the data in Table I.

The increase of total salts, which are mostly *'kallar'* salts, namely—carbonates, bicarbonates, chlorides and sulphates of sodium and potassium present in the 'dhand' water, is due to the addition of these salts from the seepage-water received by the 'dhand' water during these months. It is to be noted particularly that there were no rains from October 1945 to June, 1946, which could wash into the 'dhand' the *'kallar'* salts lying in large quantity in the fields on either side of the 'dhand'.

Significance of the composition of water in the behaviour of fish

It has been found out that the 'dhand' contains no adult *Calla* from October to June. The adult fish in the river are attracted into this 'dhand' due probably to the presence of brackish water in the 'dhand', when the level of river-water rises in June-July. They lay their eggs and then leave

the 'dhand' by the month of September when the level of river-water falls down. *Catla* fry of very small size (half an inch in length) were seen in large numbers in a part of the 'dhand' during the last week of June, 1946. The tiny *Catla* fry grow and live in Khakhra 'dhand' from the time of hatching till the next year. Most of the fry die in April, if not removed earlier. The death is brought about due to the lack of oxygen in the 'dhand' water when decomposition of the organic matter takes place. Most of the algae and other aquatic plants, viz., *Potamogeton pectinatus*, die during April on account of the high concentration of salts in the 'dhand' water. By then, the fry or the fingerlings which happen to survive, become ten to twelve months old, and when fresh water from the river enters the 'dhand' during the month of June, the yearlings leave the 'dhand'. We get a fresh supply of the fry from the eggs laid by *Catla* and other carps during the months June-July.

Adult *Catla* of the river, most probably, enter the 'dhand' due to their liking for the brackish water, when the water-level of the river rises. The fields lying in between the river and the 'dhand' serve as spawning ground for the adult *Catla* during the flow of water from the river to the 'dhand'. The continuous supply of fresh water from the river keeps the temperature of 'dhand' water sufficiently low, and helps the tiny fry, to a great extent, in its struggle for existence.

SUMMARY

Due to the restricted distribution of *Catla* in the rivers and streams of the Punjab, its spawning has not so far been studied. The presence of *Catla* fry in large numbers in certain 'dhands' and pools is an indication of the spawning of *Catla* in those places. *Catla* is not known to breed in confined waters.

Catla fry formed 60 per cent of the fish population of Khakhra 'dhand'. It is not found anywhere else in such a great number throughout the Punjab. Adult *Catla* is not present in this 'dhand' before the spawning season. The spawning of *Catla* in this place is independent of the local rains. It takes place during the month of June or July. The fields lying in between the river and the 'dhand' are inundated when the level of Sutlej river-water rises due to the melting of snow and rains in the hills.

Catla present in the river are most probably attracted by the saline water of the 'dhand', and during their journey from the river to the 'dhand' they spawn in the inundated fields. The spent-up *Catla* leave the 'dhand' when the water from the 'dhand' and the inundated fields recedes.

The temperature of the 'dhand' water remains suitable for the spawning of *Catla*, as it is kept low by the inrush of fresh water from the river. The low temperature of the 'dhand' water also helps the tiny fry in their struggle for existence during the hot months.

DETECTION OF ARSENICAL POISONING IN AN EXHUMED CARCASE

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(Received for publication on 1 March 1948)

ARSENICAL poisoning in animals as well as in human beings is quite common, but a record of cases in animals where the carcases have been exhumed are rare or almost absent. The object of this note is to record one such investigation. It was reported in the Indian Express of 23 September 1947, that a thousand head of cattle had died of poisoning in Ottapalem area in Malabar District but on confirmation, however, it was found that only cattle worth 1,000 rupees had died. Arsenical poisoning of malicious nature was suspected because of the value of the skins that would become available on death of animals. It was stated that after some deaths, carcases were buried, thereby preventing the interested parties for having access to the skins and that, this precautionary measure resulted in the stoppage of such deaths. No detailed investigation was intended because it was thought that the carcases would be several days old and that it would be difficult to make out any organs or structure or to get material for chemical analysis. Mr Hurley, the Director however considered that it would be useful to conduct one or two postmortem examinations since arsenic is suspected and that the carcases might still be in a state of preservation.

History

Twenty two animals had died after an illness of one to two days duration in the course of about a month. No occurrence of any contagious disease during that period was reported. The symptoms mentioned by the ryots were highly suggestive of arsenical poisoning. The poison was said to be concealed in jack fruits left in the grazing areas, for the animals to ingest, while grazing.

Postmortem

The carcase of an animal that had died most recently was selected and examined. The animal was a dark brown bullock—five years old. It was reported that the symptoms that the animal exhibited were uneasiness, colicky pains, profuse diarrhoea which later on became mucus-coated and eventually blood stained. The symptoms lasted for about 28 hours. Just before death prostration, violent kicking, of the legs, striking of the head from side to side as if in acute pain was noticed. Anus became everted and the animal strained violently. The interval between death of the animal and the postmortem was roughly calculated to be about six weeks.

External appearance

On exhumation, the carcase was seen lying on its left side. The earth covering the carcase was found charred for about half an inch depth all-round. The skin with hairs was found in tact. No external injury was noticed. Decomposition was only partial and the odour was not offensive. It smelt like fermented aloes. Neither were any blow flies attracted, nor any maggots had formed. The hairs came off the skin only after a light pull. The abdomen was found contracted and there was a perceptible depression at the flanks. On the whole, the general appearance of the carcase was one of mummification. Rigor mortis had passed away. Eyelids were open and eyeballs tamified. There was no discharge at the mouth and nostrils. The tongue still retained its colour and consistency while the skeletal muscles had undergone partial decomposition and softening.

Internal appearance

There was present moderate subcutaneous fat. Peritoneumx was dried up and the position of the organs in the abdominal cavity was normal. The heart had contracted to one quarter of its

usual size and the auricles and ventricles were empty. Blood vessels were normal. Lungs decomposed and partly liquefied. Nothing unusual was noticed in trachea. Larynx and glottis. Liver was contracted and hardened with capsules intact; consistency was a little harder than normal and gall bladder was empty. Spleen was highly decomposed and jelly-like, but retained its form and size with its capsule intact. Kidneys were jelly-like and decomposed. Oesophagus was also decomposed. The four compartments of the stomach were still intact and could be separately made out. Rumen contained the usual amount of ingesta in an almost dried up state with the usual colour. The contents of the other three compartments were drier than normal and decidedly darker. The inner lining of the three compartments was black. Small intestines were contracted and tumified, almost empty; and mucus lining was darker than normal. Large intestines were also contracted and tumified and contained hard dry dung. Nothing unusual was noticed in the lymphatic glands, bladder or generative organs. Brain and spinal cord were liquefied and jelly-like.

It was, thus obvious, that the carcase was contracted and in mummified condition and the skin and the hair were intact. There was absence of postmortem changes in the tongue which was normal in colour and consistency. The liver was contracted and hardened and seemed to be in a fair state of preservation. The condition of the heart and stomach and the intestines also signified that they were contracted and tumified and not decomposed. There was absence of maggot formation.

Diagnosis

Arsenical poisoning was suspected. The contents of stomach, including that of the rumen and bits of liver and intestines preserved in rectified spirit with a control sample of rectified spirit, were sent to the Chemical Examiner, who detected arsenic in all the samples, except the control one.

Discussion

As stated in the beginning, the cases of malicious arsenical poisoning of cattle have been frequently met with, in the past in this country. The chief motive is the procurement of the skin, though ill feelings, personal jealousies and quarrels among villagers often play a part. The crime is invariably perpetrated by the *Chamars* or chucklers either on their own initiative or at the instigation of others. The other poisons used are mercury, and vegetable, but arsenic is the most commonly used because of the smallness of its dose and the convenience of administration, and easy availability.

According to Hehir and Gribble [1929] there were 283 cases of arsenical poisoning out of the total of 293, during the five years from 1885 to 1889.

Cases of exhumation are comparatively rare or even absent. In cases of human beings there are quite a few records specially where the bodies have been buried either enclosed in a coffin or free. In animals there are rare cases of burial or cremation, because of the usual practice of skinning the carcase and discarding the remains. From a reference to the statistics available with the Chemical Examiner to the Government of Madras from 1942 to 1946, 15 cattle and 23 human beings were diagnozed against arsenical poisoning and except for one case in human beings, no carcase was exhumed.

In passing, the preservative qualities of arsenic may be mentioned. Macfall [1925] has mentioned in this connection that arsenic is an indestructible poison and may be found in the body after many years; in one case it was found after 14 years. Whitford [1884] records, that the body in one case was found remarkably preserved for a period of 37½ months after interment. Jai Singh and P. Modi [1945] state that the body of Full Ham of Agra was found to be well preserved when it was exhumed 14 months after death, even though the grave was a kutcha one and the lid of the coffin had already given way.

Apparently, in the carcase of the bull exhumed, it was the preservative effect of the arsenic that had resulted in the prevention of putrefaction and preservation of some of the organs.

The appearances met with, in the different organs, are very varied and interesting, particularly indicating the relation to their distribution of arsenic in the various tissues and body fluids. While the skin with hairs, tongue, heart, liver and all the four compartments of the stomach and intestines were in the state of fair preservation, the skeletal muscles, lungs, spleen, kidneys and brain had

undergone decomposition and complete softening approaching liquefaction. John Glaister [1945] has mentioned in this connection, that arsenic quickly absorbed is stored in the liver and from there it passes to general circulation. Although the intestinal tract eliminates some of the arsenic from the body the kidneys are the principal excretory organs. From this it is evident that the liver is the main reservoir of the chemical, from where it passes on to the kidneys and intestines through circulation. The following Table from Glaister [1945] gives the distribution of Arsenic in the various tissues and body fluids.

TABLE I

Distribution of arsenic in the various tissues and body fluids

Tissues	Total weight received	Arsenic in tissues	
		Arsenic in total weight in grains	Arsenic as part per million
	Lbs. Ozs. drs.		
Brain	3 1 0	0·18	·9
Stomach tissue	0 13 0	·143	25·0
Stomach contents	0 14 0	5·18	861·0
Intestinal tissue	3 6 0	1·29	54·0
,, contents	1 12 0	5·88	486·0
Liver	4 3 0	2·33	80·0
Spleen	0 4 0	0·009	5·8
Kidney	0 9 0	0·103	27·0
Pancreas	0 4 0	0·002	1·2
Heart	0 12 0	0·011	2·0
Adrenals	0 0 6	0·004	9·6
Lungs	1 3 0	0·026	3·2
Urine	0 0 6	0·004	15·6
Bile	0 1 0	0·003	5·7

From the above Table I it is seen that the highest concentration is in the stomach contents next in order the intestinal contents, liver, intestinal tissue, kidneys and stomach tissue. In all the above organs and their contents the concentration ranges from about 25 to 860 parts per million. In other tissues and fluids the concentration is below 15 parts per million.

It will thus be observed, that the degree of preservation in the different organs in this case is in direct proportion to the amount of arsenic present except in the case of kidney, which was perhaps putrified due to the acute nature of the poisoning, as evidenced by the sudden death, which might have prevented the usual amount of the chemical going to the kidneys.

SUMMARY

A case of poisoning of cattle with arsenic, where the carcase was exhumed after a period of six weeks is recorded. The organs, like the liver, stomach, intestines, which are likely to contain a considerable proportion of arsenic were found in a state of preservation.

ACKNOWLEDGEMENTS

I am grateful to Mr Hurley for his valuable suggestion, to Mr C. Madhavan Nambiar and his staff for their cooperation and help and to Dr K. C. Jacob, Professor of Medical Jurisprudence and Dr P. Venkat Rao, Chemical Examiner for having placed the necessary literature at my disposal.

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THE EFFECT OF LATE NITROGENOUS TOP-DRESSING ON THE DIGESTIBILITY OF HAY

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[Reprinted from *The Journal of Agricultural Science* Vol. 38 No. 1 January 1948]

The application of soluble nitrogenous fertilizers to a hay crop some 7-20 days before the crop is cut results in an increase in the protein content of the hay.

In 12 experiments, Lewis [1941] showed that 1 cwt./acre of sulphate of ammonia, nitrate of soda or 'Nitro-Chalk' gave an average increase of 1·0 per cent in the crude-protein content and with 2 cwt. fertilizer/acre the average increase was 1·75 per cent. He also showed that most of the extra nitrogen absorbed by the crops was converted into true protein and, with two minor exceptions, the proportions of nitrates and ammonia were undisturbed.

Later Lewis [Jealott's Hill Report, unpublished] summarized the results obtained in 47 replicated experiments carried out in various parts of England and northern Ireland between 1938 and 1946. The nitrogen dressing used varied slightly. From 1935 to 1945, 1 and 2 cwt./acre 'Nitro-Chalk' or sulphate of ammonia were applied and in 1946 1¼ and 2½ cwt./acre 'Nitro-Chalk'.

The mean results obtained are given in Table I.

Table I

*Crude protein in hay (per cent)**

—	No late N dressing	Single N dressing	Double N dressing
1938-45	8·92	10·02	10·98
1946	7·30	8·30	8·71
All trials	8·32	9·43	10·11

*Moisture content 15 per cent

The mean of the rations (true protein)/(crude protein) were :—

—	Mean	Range
No late N dressing	0·83	0·70—0·91
N dressing	0·81	0·63—0·90

Single and double dressings of nitrogen fertilizer resulted in increases of 13 and 23 per cent respectively in the crude-protein contents of the hays, and the extra nitrogen absorbed was mainly elaborated into true protein.

The rapid rate at which the absorbed nitrogen is converted into 'true' (copper precipitable) protein is of considerable interest, but direct evidence is needed as to the nutritional value to farm animals of such protein. A direct comparison of the amino-acids present in late top-dressed hay and hay not top-dressed might yield circumstantial evidence on the nutritive value of the added protein, but no work on this line has been attempted. A comparative feeding trial using dairy cows or young stock is obviously desirable, but in view of the relatively small difference to be measured, a high or practically unattainable degree of accuracy would be necessary. The obvious necessity of a ration of minimal protein content adds to the difficulties of such a feeding trial.

Since the major part of the added nitrogen is present in a form precipitable by alkaline copper hydroxide and therefore might be expected to be protein, polypeptides or certain amino-acids, it is thought that analysis supported by information on the digestibility of the protein should give a reasonable estimate of nutritive value of the protein in top-dressed hay. Such information is presented in this paper.

Experimental

The hay was from a ley in its third year. It was a good crop consisting mainly of vigorous tye-grass with some leafy cocksfoot. The clover content was fairly low. A block of about 2 acres was top-dressed with 2 cwt. sulphate of ammonia, per acre on 31 May and cut on 13 June. Rain fell on the 11th day after the dressing, the total rainfall being 1·33 in. The weather was cool with bright intervals. No rain fell after the cutting, and the top-dressed and control hays were baled in the field on 19 and 20 June. The crop, and also the stubble after cutting, were noticeably greener on the top-dressed area. The digestibility of these hays was determined using three sheep for each hay.

In 1946 the hay was made from old permanent grass, the herbage consisting mainly of meadow grasses with a little wild white clover. The botanical composition varied considerably over the field.

The lay-out consisted of eight strips, each about 33 ft. wide, running the length of the field. Alternate strips were top-dressed with 3 cwt. 'Nitro-Chalk' per acre. Yields of hay were measured, but as expected the top-dressing resulted in no increase in yield. Top-dressing was applied on 13 June and the crop was cut on 21 June. The hay was cocked on 27 June and baled on 1 July.

Slight rain fell each day between 13 and 21 June, the total rainfall being 0·8 in. A further 0·9 in. of rain was recorded in 5 days between 21 and 28 June.

The aftermath on the treated strips was markedly greener than that on the control strips.

Digestibility trials were carried out on the two hays using four sheep for each hay.

Results

Chemical composition of the hays

The hays were sampled by selecting 10-12 bales at random from the main crops. These bales were opened and 7 lb. hay removed from two places in each bale. The samples were then chaffed, thoroughly mixed and used for the digestibility trials. Daily sub-samples were taken during the 10-day digestibility trials and these were composited for analysis.

The compositions of the hays are given in Table II.

TABLE II

Composition of hays as percentage of dry matter

	1945		1946	
	Top-dressed	Control	Top-dressed	Control
Ether extract	1·79	1·57	1·32	1·32
Fibre	32·17	32·48	36·05	36·30
Crude protein	11·57	9·67	12·41	10·36
Ash	7·19	7·16	8·13	7·99
N-free extract	47·28	49·12	42·09	44·03
	100·00	100·00	100·00	100·00
Organic matter	92·81	92·84	91·87	92·01
True protein	9·87	8·69	10·68	9·46
Ratio $\frac{\text{True protein}}{\text{Crude protein}}$	0·85	0·90	0·86	0·91

As would be expected the top-dressing caused little change in composition, the increase in protein being balanced mainly by a fall in N-free extract.

The rations true : crude protein show a slightly greater proportion of non-protein nitrogenous compounds in the top-dressed hays. Top-dressing resulted in increases of 1·18 and 1·22 per cent of true protein in the 1945 and 1946 hays respectively compared with increases of 1·90 and 2·05 per cent of crude protein. The quantities of nitrogen, expressed as crude protein, absorbed but not elaborated into 'true' protein were therefore 0·72 and 0·83 per cent respectively. The 1946 hays were analysed for nitrate and ammonia content with the following results in Table III.

TABLE III

Analysis of 1946 hays

	Top-dressed hay	Control hay
Ammonia N, per cent dry matter	0·165	0·120
Nitrate N, per cent dry matter	0·037	0·025
Ammonia N as per cent of total N	0·83	0·72
Nitrate N as per cent of total N	0·19	0·15

The top-dressed hay was higher in both ammonia and nitrate contents but the differences were small.

Digestibility of hays

A statistical lay-out for the digestibility trials was adopted in both years. Three she used on each hay in 1945 and four sheep in 1946.

In both years the agreement between sheep was satisfactory. As is seen from the individual digestibility coefficients of the hay constituents given in the Appendix. The mean values are given in Table IV.

TABLE IV

Digestibility coefficients of hay constituents (percentage)

	1945		1946	
	Top-dressed	Control	Top-dressed	Control
Dry matter	60·8	60·3	58·6	57·2
Organic matter	62·5	62·3	60·6	59·2
Ether extract	41·6	41·2	23·5	23·3
Fibre	63·9	65·3	71·6	70·1
N-free extract	63·4	63·6	54·3	54·5
Crude protein	58·3	48·9	53·8	45·4
True protein	54·2	46·6	49·5	43·3

Comparing the top-dressed and control hays the digestibilites of the constituents were remarkably similar except for the protein. Both crude and true protein were markedly more digestible in the top-dressed hays. The increase in digestibility of the true protein due to top-dressing is of particular interest.

In both years, as will be seen from the figures given below, the digestibility of the true protein in the top-dressed hays was significantly higher than in the control hays. In 1946 the difference was significant at the 100 : 1 level of probability.

	1945	1946
Top-dressed hay (adjusted for block differences)	53·5	49·5
Control hay (adjusted for block differences)	46·9	43·3
Significant difference : P = 0·05	6·3	3·9
P = 0·01	..	5·9

Nutritive values of the hays

The nutritive values of the hays, expressed as starch and protein equivalents on a dry-matter basis, are given in Table V.

TABLE V

Starch and protein equivalents of hays, on dry matter (percentage)

1945 hays	Starch equivalent	Protein equivalent
Top-dressed	37·8	5·97
Control	38·6	4·42
Significant difference (P = 0·05) 1946 hays	..	0·66
Top-dressed	33·3	5·98
Control	32·9	4·40
Significant difference (P-0.95)	..	0·38

The values have been adjusted for block differences, and comparison with one mean figure quoted in the Appendix shows these adjustments were very small.

There were obviously no significant differences between the top-dressed and control hays as regards starch equivalent values, but the differences in protein equivalent values were highly significant. These were very similar in both hays and the top-dressing resulted in increases of 1·55 and 1·58 per cent protein equivalent.

Throughout this paper the data have all been expressed on a dry-matter basis, and certain figures may be useful expressed on hay containing the average dry-matter cotent of 85 per cent given in Table VI.

TABLE VI

	1945 hays			1946 hays		
	Top-dressed	Control	Increase	Top-dressed	Control	Increase
Crude protein	9·83	8·22	1·61	10·55	8·81	1·74
True protein	8·39	7·39	1·00	9·08	8·04	1·04
Digestible crude protein	5·73	4·01	1·72	5·67	4·00	1·67
Digestible true protein	4·55	3·44	1·11	4·50	3·49	1·01
Protein equivalent	5·14	3·73	1·41	5·08	3·74	1·34
Starch equivalent	32·3	32·9	—0·6	28·3	27·9	0·4

DISCUSSION

If the increases of total and digestible crude and true protein due to top-dressing are compared it is seen that the figures are almost identical:

Increase due to top-dressing (per cent)

	Total	Digestible
Crude protein: 1945	1·90	2·02
1946	2·05	1·97
True protein: 1945	1·18	1·30
1946	1·22	1·19

This suggests that either the extra protein was entirely digestible or it improved the digestibility of the total protein present.

It seems likely that this would obtain whatever response to top-dressing was obtained, providing the crop showed no marked increase in growth due to the top-dressing.

In the present experiments the response to nitrogen was good—about 2·0 per cent crude protein, 1·2 per cent true protein and 1·6 per cent protein equivalent, all on the dry matter. The two hays gave almost identical results although one was a good ley mixture and the other a rather

poor permanent grass, and it might be tentatively suggested that an increase of 2·0 per cent in crude protein would generally give an increase of about 1·6 per cent in protein equivalent. Whether the ratio, increase of crude protein : increase of protein equivalent, 1 : 0·8, would hold whatever the response to top-dressing remains to be proven, but this does not appear unreasonable.

The protein contents of the hays in this country are generally distressingly low and methods of increasing the level are desirable. The present work shows that a useful increase in 'apparent' digestible protein results from late nitrogenous top-dressing. Even if part of the nitrogen is not elaborated to protein in the plant it may still have appreciable feeding value for ruminants in view of the latter's ability to utilize non-protein nitrogenous substances.

An additional benefit from the top-dressing is the stimulating effect on the growth of the aftermath and it appears therefore that late top-dressing of the hay crop is a valuable practice which might be more generally applied.

Appendix

Digestibilities of hay constituents

—	Sheep	Dry matter	Organic matter	Ether extracts	Fibre	N-free extract	Crude protein	True protein
					1945 hays			
Top-dressed	1 .	60·6	62·2	44·0	64·4	62·5	57·9	53·4
	6 .	60·7	62·8	39·7	64·2	64·2	55·7	51·8
	10 .	61·2	62·7	41·2	62·9	63·6	61·4	57·4
	Mean	60·8	62·5	41·6	63·9	63·4	58·3	54·2
Control	3 .	60·7	62·5	45·0	66·6	62·7	50·9	49·3
	4 .	61·3	63·5	43·6	65·5	65·8	48·2	45·5
	9 .	58·9	60·9	35·0	63·8	62·4	47·5	45·0
	Mean	60·3	62·3	41·2	65·3	63·6	48·9	46·6
					1946 hays			
Top-dressed	2 .	57·5	59·0	22·0	69·7	52·0	55·3	51·6
	4 .	58·4	60·9	22·0	72·7	54·9	51·7	47·5
	8 .	59·3	61·2	22·0	71·9	55·2	54·5	49·6
	10 .	59·3	61·2	28·0	72·1	55·3	53·6	49·3
	Mean	58·6	60·6	23·5	71·6	54·3	53·8	49·5
Control	1 .	56·9	58·1	23·0	68·4	54·0	46·5	45·5
	3 .	56·5	58·4	29·0	68·2	53·7	48·0	45·5
	7 .	56·5	58·4	14·0	70·6	52·9	44·4	41·9
	9 .	58·8	61·4	27·4	73·2	57·2	42·5	40·1
	Mean	57·2	59·2	23·3	70·1	54·5	45·4	43·3

APPENDIX—*contd.*

Digestible nutrient content of hays

		Digestible ether extract	Digestible fibre	Digestible N-free extract	Digestible crude protein	Digestible true protein	Protein equivalent	Starch equivalent
				1945 hays				
	Sheep							
Top-dressed*	1 .	0·78	20·7	29·6	6·69	5·27	5·98	38·08
	6 .	0·71	20·6	30·4	6·44	5·11	5·78	38·51
	10 .	0·74	20·3	29·0	7·10	5·66	6·38	37·33
	Mean	0·74	20·5	29·6	6·74	5·35	6·05	37·97
Control	3 .	0·71	21·6	30·8	4·92	4·28	4·60	38·96
	4 .	0·68	21·3	32·3	4·66	3·95	4·31	39·78
	9 .	0·55	20·7	30·7	4·59	3·91	4·25	37·26
	Mean	0·55	21·2	31·3	4·72	4·05	4·39	38·67
				1946 hays				
Top-dressed	2 .	0·29	25·1	21·9	6·86	5·51	6·19	31·82
	4 .	0·29	26·2	23·1	6·42	5·07	5·75	33·70
	8 .	0·29	25·9	23·2	6·76	5·29	6·03	33·75
	10 .	0·37	26·0	23·3	6·65	5·27	5·96	33·98
	Mean	0·31	25·8	22·9	6·67	5·29	5·98	33·31
Control	1 .	0·30	24·8	23·8	4·82	4·31	4·57	32·21
	3 .	0·38	24·8	23·7	4·97	4·31	4·64	32·13
	7 .	0·18	25·6	23·2	4·60	3·96	4·28	31·96
	9 .	0·36	26·6	25·2	4·40	3·80	4·10	35·23
	Mean	0·31	25·4	24·0	4·70	4·10	4·40	32·88

SUMMARY

Comparative digestibility trials have been carried out on hays top-dressed with nitrogenous fertilizers 8-13 days before cutting and on similar hays which were not top-dressed.

The top-dressing resulted in an increase of 2·0 per cent crude protein and 1·2 per cent true protein.

The digestibility of the protein was significantly higher in the top-dressed hays and no other constituents were affected.

The protein equivalents in hay, dry matter were increased from 4·4 per cent to 6·0 per cent by the top-dressing.

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ABSTRACTS

Jacotot, H. (1947). **Etude du vaccine contre la peste bovine.** *Rev. d'Immun. Ther. Antimicrob.* **11**, 4-5, 222-275 [English summary]

(Author's conclusions)

THE normal saline wash of minced tissue constitutes a poor vaccine in general. Its efficacy is proportionate with the quantity of antigen extracted by the processes of mincing and washing by gathering this small quantity of antigen one does not obtain an increase of vaccinating substance.

Formalinized dried vaccine is superior to formalinized liquid vaccine even when the latter is freshly prepared; the first vaccine best conserves its potency at 25-30°C. and the latter at 6°C.

Formalinized dried vaccine maintained at laboratory temperature is somewhat inferior to toluene vaccine maintained under the same conditions.

The immunity conferred by the dry vaccine is almost of the same duration as the immunity conferred by toluene vaccine but the results of vaccination are probably more uniform with the former; dry formalinized vaccine gives a more lasting immunity than the ordinary liquid formalinized vaccine.

In the same conditions of concentration of the virus, whether it be in the form of a powder or a saline extract, the dry vaccine has a much smaller volume; this is an advantage which favours its use in certain circumstances. However, such circumstances are not always encountered in practice; particularly in Indo-China vaccination in the field has to be carried out by subordinate staff to whom the vaccine is sent from the nearest veterinary field dispensary. There is an additional disadvantage in that the dry vaccine forms clumps when water is added to the powder and this not only makes vaccination difficult but also the assessment of the actual quantity of the vaccine inoculated. This is the reason for our present opinion that a formalinized adsorbed alumgel vaccine is much to be preferred in field practice. [J. F. S.]

Jacotot, H. (1947). **Etude du vaccine contre la peste bovine** (2nd memoire). Les techniques vaccination. *Rev. d'Immun. Ther. Antimicrob.* **11**, 6, 329-381.

(Author's summary and conclusions)

THERE is no reason to doubt or even question the conclusion that rinderpest vaccines prepared from formalinized emulsions of the tissues of a bovine experimentally or otherwise injected with the virus of rinderpest contains living virus; such vaccines nevertheless possess strong and uniform immunizing properties. Other things being equal, such as the methods of preparation of the vaccines and the actual concentration of tissues in the emulsion, vaccine prepared from lymph gland tissue is approximately twice as potent as that prepared from spleen pulp and at least four times as active antigenically as that made from lung pulp. Even the intestinal mucosa may be used in vaccine preparation, the necrotic portion especially having a higher antigenic value than spleen pulp. The small pox lymph obtained from she buffaloes or heifers inoculated both with rinderpest virus and lymph vaccine possesses an antigenic value almost comparable with fresh rinderpest virus and of greater potency that spleen pulp. Liver and testicular tissues are antigenically inactive or at the best have but feeble immunizing value and are valueless in the preparation of rinderpest vaccine.

The antigenic value of the selected tissues of calves artificially infected with virus is *nil* during the period of inoculation; it is evident, though very feeble, at the first day of fever and reaches its maximum with the maximum temperature reaction, or very shortly after; it remains at this level for two or three days then diminishes until at about the tenth day it is hardly demonstrable.

There is a relationship between the antigenic value of the tissues and their actual virus content but it is probable that they owe their antigenic properties to the manner in which the virus is attached to certain component cells. It is not necessary that an animal inoculated with rinderpest virus should show a reaction so that the tissues are suitable for vaccine production. The antigenic value of those tissues is as good as in animal reacting fully. The deterioration of rinderpest vaccine is shown both by its failure to produce immunity and the irregular reaction in vaccinated animals. A low storage temperature is fundamentally necessary in the conservation of the full antigenic potency of emulsion vaccines. A toluine vaccine gives better results than a formalinized vaccine. It possesses stronger immunizing value, especially marked when freshly prepared, and antigenic qualities are better maintained in storage. Dried formalinized vaccine is better than liquid and retains its full potency better. It is somewhat inferior as a vaccine compared with toluine vaccine but it has the advantage of a small bulk and this balances, in practice in the field, its somewhat poorer antigenic value. It does not appear that the immunity following two vaccinations is any better than a single vaccination but the advantage of the former method is definitely apparent in the building of a stronger immunity in the week's following vaccination. The administration of the large quantity of vaccine notably prolongs the duration of immunity but after a period of eight months the benefit of massive inoculation is not so obvious. Comparing the results of vaccination with formalinized, toluine, and flourine vaccines inclines one to the belief that a relationship exists between the reaction at the site of inoculation and the duration of immunity conferred by the inoculation. The fact that a vaccinated animal can withstand a moderate test dose of virus does not infer that it can tolerate without severe reaction a larger test dose. An animal which has failed to react to several small test doses of virus will certainly react strongly to a large dose of the same virus. The quality of the vaccine, the dose given, the time elapsing after vaccination, the size of the test dose are, however, factors that operate in immunity. In animals vaccinated and tested by standard methods, 10-20 per cent of them remain or later become susceptible to infection with rinderpest virus. The antigenic value of a virulent pulp vaccines is generally feeble. The intravenous inoculation of a vaccine has no prophylactic value; given in the incubation phase or in the preliminary febrile phase the vaccine will not avert the normal development of the disease. Consequent to vaccination with massive doses of formalinized vaccine the serum of some young cattle acquires a strongly prophylactic value. Young calves, the offspring of highly immunized cows, may be successfully immunized by means of a virulent spleen and other tissue extracts; there is no incompatibility between the immunity of maternal origin which lasts until weaning and that resulting from a later vaccination.

The addition of aluminium hydroxide to formalinized suspensions is an improvement. Vaccine adsorbed on aluminum gel gives a solid immunity with a tenth of the dose of the ordinary formalinized vaccine used immediately on preparation and it also retains its antigenic value longer. Thus, the ratio of activity at the first test is 1/10, 1/15, 1/20 and on retest two to three months later 1/20, 1/30, 1/40, provided that both vaccines under test have been subject to optimum storage temperatures. Absorbed vaccine possesses when just prepared and even on storage a uniformity of antigenic value that the other vaccine never possesses, even when freshly prepared. Tissue vaccines treated with aluminum gel are comparable in their antigenic power with ordinary tissue suspension vaccines, but their effect is slightly more durable. The increase of antigenic potency of the vaccine is directly related to the quantity of adjuvant incorporated in the vaccine; 60 parts of pulp mixed with 100 parts of gel equivalent to 5·5 gm. aluminum hydroxide is the most satisfactory working ratio and fulfils all practical needs. The gel seems to exercise a double roll; it stimulates the development of immunity (action on the tissues) and protects the formalinized pulp against deterioration (action on the vaccine).

Between the years 1940-45, 55,000,000 c.c. gel rinderpest vaccine have been used in Indo-China and at least 2,000,000 cattle have been vaccinated. Wherever it has been properly used, the adsorbed vaccine has given the results expected of it. Of the practical results following the use of this vaccine, mention must be made of the saving of young cattle used for the provision of vaccine material, a ratio of almost 1/3 compared with the preparation of other tissue vaccines. [J. F. S.]

Ralph W. Phillips, Victor L. Simmons & Ralph G. Scohott. (1943.). **Observations on the normal estrous cycle and breeding season in goats and possibilities of modification of the breeding season with gonadotropic hormones.** *Amer. Jour. Vet. Res.* 4,360-367.

THE observations recorded in the paper were made with a view to the production of milk throughout the year as the breeding season in goats is limited and goats milk only for five or six months during a lactation.

To achieve this and certain possible means that can be exploited are: (a) Does may be bred at different times during the breeding season, (b) Select such does that come in estrus out-side the breeding season, (c) To bring about estrus by artificial means such as treatment with gonad stimulating hormones and, (d) Stimulation of lactation without gestation by use of lactogenic hormones.

Literature has been quoted where extra seasonal breeding in goats have been induced by reduction of light during the early part of summer and inducing lactation in virgin goats by injections of stilbestrol.

In Swiss goats ovulation occurs during the months of October, November and December but may extend to other periods due to domestication ovulation occurs at regular intervals of three weeks. Whereas in Boer and Angora goats in S. Africa the breeding season is from April to August.

The authors observations were mainly on the duration of breeding season, the length of estrous cycle and on the possibility of obtaining pregnancies in does outside the breeding season by means of gonadotropic hormones.

Saanen and Toggenburg does and kids were used in study. The total number observed for estrus included 32 milking does, 20 dry does, 16 kids born in 1941 and 19 kids born in 1942. The observations started from 20th October 1941 and lasted up to 13 December 1942. These observations indicate that most does will come in estrus between the middle of September and middle of December. A few, during July, August and early September and some in April, May and June.

Estrous cycles were observed in milking and dry does and in the 1941 kids. A considerable number of estrous cycle fall in about 21 days. Shorter cycles of 12 days or less as well as longer ones which were multiples of 21 days were also observed. The average length of estrous cycles of 161 observations was 22·8 and 23 days in dry and milking does respectively and 16·6 days in kids.

The results of hormone treatment with P. M. S. (pregnant mare serum) indicate that one injection of 400 units will bring about estrus in dry does whereas lactating does require 400-600 units and even then they may not respond as readily as dry does. Of the 15 dry and 4 lactating does which came in estrus as a result of treatment with P. M. S. only six dry and one lactating doe conceived and produced kids though all were bred while in estrus. The number is too small to arrive at a definite conclusion. A small trial with luteinizing hormone indicated that it may be helpful in stimulating ovulation at the proper time. [M. K. S.]

Schofield F. W. & Barnum, D. A. (1947). Limitations in the use of Penicillin in the Treatment and Eradication of Bovine Mastitis. *Jour. Amer. Vet. Med. Assn. Oc.* **92-96.**

PENICILLIN, the wonder drug, which enjoyed a period of unstinted praise as a drug of great value in the treatment of bacterial diseases in man and animals was found to have unanticipated limitations when more comprehensive and exacting tests were made with it in certain diseases. In this article, the authors deal with some of the limitations of penicillin in the treatment of bovine mastitis. In bovine mastitis it is necessary to determine the nature and type of infection by bacteriological methods before penicillin therapy is adopted. If the drug is used indiscriminately it will rapidly bring it into disrepute. In certain infections penicillin will have to be combined with other methods of treatment.

The authors used penicillin in two outbreaks of acute mastitis due to *Streptococcus agalactiae* characterized by sudden onset and rapid spread of the disease terminating in atrophy or fibrosis of

the affected quarter and reduction of milk flow. In this type of infection the organism quickly penetrate the udder-parenchyma and as such penicillin fails to contact them effectively. It was found that from the stand-point of sterilizing the udder, in such cases penicillin was found to be disappointing. Clinically, however, the treatment resulted in marked physical improvement and the owners get satisfied consequently with the good results that ensue. The authors consider penicillin most effective in the treatment of chronic mastitis caused by *Str. agalactiae* where the infection is dormant. In these cases the infected quarters become free from infection and remain so for months and the milk yield increases in some cases.

In the treatment of mastitis due to *Staphylococcus aureus* and *Coryne-bacterium pyogenes*, the efficacy of penicillin is dependent upon its immediate use in the early stages. In the later stages of these conditions, penicillin is valueless. [P. R. K. I.]

Minett, F. C. (1947). **Effects of artificial showers, natural rain and wallowing on the body temperature of animals.** *J. Ani. Sci.*, 35-39.

THE author carried out experiments to find out the extent of body heat loss by body wetting in buffaloes, zebu cattle, small hill cattle and sheep. The wetting methods employed were artificial showers, natural rain, wallowing, hosing and splashing. Chilling affects the well-being of animals which explains the incidence of certain diseases during monsoon in India.

A two hour's heavy shower in the morning lowers the body temperature of buffaloes by 2·8°F. and a similar shower in the afternoon lowers the body temperature of buffaloes and cows by 1·6°F. and 0·5°F. respectively. The fall in buffaloes is during the shower and in cows during subsequent drying. Young buffaloes suffer more than adults. The body temperature fall under natural shower ranges between 0·7° to 2·7°F. in cows, 1·6 to 3·3°F. in hill cattle and 1·4° to 2·7°F. in sheep. The results clearly show the inefficiency of temperature control in the buffalo as compared with the zebu cow which is probably due to the protective coat in cows and the less efficient hyroid adrenal mechanism in buffaloes.

Body cooling or wallowing is indispensible for the buffalo in summer. Inclination to wallow is intense from July to October. Body cooling by hosing for three minutes is as good as wallowing for 20 minutes or one hour, but splashing for 10 minutes is not as effective. Wallowing by a previously-exercized animal reduces the body temperature by 3° to 4°F. The author recommends showers in place of wallows for organized dairies. [K. C. S.]

George D. Quigley & Earnest N. Cory. **The utility of D. D. T. for the control of poultry ectoparasites.** *Poultry Sci. Sept.* 1946, Vol. XXV, No. 5, 419-423.

WHEN 50 mg. of D. D. T. was fed mixed with all mash ration even up to 120 days, no toxic effects were evidenced but apparent elimination of body lice took place after 10 weeks. Eggs from treated birds were not toxic to experimental rats upon feeding, but when the same amount of D.D.T. was given by mount in capsules toxic effects were noticed after 20 days, suggesting the cumulative nature of the drug. Massive doses of D.D.T. though toxic in effects, did not prove lethal.

At 20 per cent and one per cent levels D.D.T. in pyrophyllite when dusted on half the egg towards the larger end or one per cent. D. D. T. in triton-xylol emulsion when applied to the egg, did not reduce hatchability.

When applied to individual birds at 2 per cent and 10 per cent levels in pyrophyllite by salt shaker method, D.D.T. gave protection for 30 days but 48 hours had to elapse before the birds were free of the lice.

Dusting of layers upon the roosts twice at least two weeks apart at 20 per cent, level in pyrophyllite was effective to control both body and shaft lice. This method had no adverse effect on egg production.

Insects and bed bugs were easily destroyed by D.D.T. and in case such dead insects are consumed in large quantities by fowls the authors are of opinion that no harm will ensue. [S. G. I.]

REVIEW

Veterinary Helminthology and Entomology

By H. O. Monnig (*Published by Bailliere Tindall and Cox, 7 and 8, Henrietta Street, London, 3rd Edition pp. XVIII+427, 31s. 6d.*)

ZOOLOGISTS in general and parasitologists in particular will doubtless hail the appearance of the third edition of Monnig's book which is very handy work of reference for students of veterinary helminthology and entomology. The first print of this book appeared only 13 years ago and the rapidity with which the third edition had to be brought out is a clear indication of its utility and popularity. The book has been written in an easy style and the general get-up is excellent. The third edition is marked out by the additional drawings of some helminth ova and gravid segments of common tapeworms of domestic animals. Some figures in the previous editions have been replaced by the author's own drawings. The information contained in Section I forms a very useful introduction to the understanding of most of the implications of veterinary parasitology. Section II is devoted to technique, the knowledge of which is indispensable to a student of parasitilogy. References have been made to the anthelmintics such as hexachlorethane and phenothiazine and insecticides such as D.D.T. and Gammexane which have been successfully used in recent years. A fairly exhaustive note has been added on phenothiazine. In a short compass of the book of this size it is not expected that all helminth and arthropod parasites so far recorded from domestic animals should have received attention but the important ones have been dealt with. It is, however, felt that reference should have been made to parasites such as *Paryphostomum sufrartyfex*, *Pseudodiscus collinsi*, *Varestronggylus pneumonicus*, *Capillaria bilobata*, *Haemonchus similis* and *Leiperacanthus gallinarum*. *Parafilaria bovicola* and *Protostronggylus rufescens* do occur in India also. The name and date [Bhalerao, 1942] have been bracketted against *Cymbiforma indica*, which is evidently an error. Mention should have been made of Tobacco-lime dressing against warble-fly larvæ.

G. D. B.

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